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Horizon Scanning Technology Prioritising Summary

Rapid molecular assays for the diagnosis of sepsis and identification of sepsis causing pathogens

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Adelaide Health Technology Assessment

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PRIORITISING SUMMARY

REGISTER ID: **000477**

NAME OF TECHNOLOGY: **RAPID MOLECULAR ASSAYS FOR THE DIAGNOSIS OF SEPSIS AND IDENTIFICATION OF SEPSIS CAUSING PATHOGENS**

PURPOSE AND TARGET GROUP: **PATIENTS SUSPECTED OF HAVING SEPSIS**

STAGE OF DEVELOPMENT (IN AUSTRALIA):

- ☒ Yet to emerge
- ☐ Experimental
- ☐ Investigational
- ☐ Nearly established
- ☐ Established
- ☐ Established *but* changed indication or modification of technique
- ☐ Should be taken out of use

AUSTRALIAN THERAPEUTIC GOODS ADMINISTRATION APPROVAL

- ☐ Yes
- ☐ No
- ☒ Not applicable

ARTG number

INTERNATIONAL UTILISATION:

COUNTRY	LEVEL OF USE		
	Trials Underway or Completed	Limited Use	Widely Diffused
Finland	✓		
United States	✓		
France	✓		
Switzerland	✓		
Italy	✓		

IMPACT SUMMARY:

Several companies market rapid molecular tests for the diagnosis of sepsis and the identification of sepsis causing pathogens including the LightCycler® SeptiFast Test MGRADE (Roche Diagnostics) and the Prove-it™ Sepsis assay (Mobidiag Ltd, Finland). The technology would be made available through specialised pathology providers for patients suspected of having sepsis. TGA approval is not required for diagnostic tests for the detection of sepsis. Neither of these products is approved by the United States FDA. Prove-it™ Sepsis has the European CE-IVD certificate for in-vitro routine diagnostics.

BACKGROUND

Sepsis is a systemic response to a localised but serious infection which is usually bacterial in origin but may be fungal. Sepsis is clinically defined as when a patient has two or more of the criteria or symptoms for systemic inflammatory response syndrome (SIRS). These criteria include: an abnormal white blood cell count[1] of >12x*10^9/L* or <4.0x*10^9/L*; an abnormal body temperature of >38°C or <36°C; tachycardia of >90 heart beats per minute; tachypnoea or an increased rate of respiration of >20 breaths per minute; or a $PaCO_2$ (partial pressure of arterial CO_2) <32mm Hg[2] (Mancini et al 2010). Severe sepsis is defined as sepsis with sepsis-induced organ dysfunction or tissue hypodiffusion, with a systolic blood pressure (SBP) of <90mm Hg or <2 standard deviations below the normal for age or a SBP decrease of >40mm Hg. Septic shock is then defined as sepsis-induced hypotension despite adequate fluid resuscitation (Ihle 2008; Mancini et al 2010).

Guidelines for the treatment and management of sepsis recommend the prompt intravenous administration of broad-spectrum antibiotics within 1-hour of diagnosis based on symptoms as described above and before confirmation of a positive result from blood culture. However, obtaining a sample for culture is essential prior to commencement of antibiotic therapy. Samples for culture should be obtained from both a peripheral site and from any vascular device in use. Delay in the administration of antibiotics is associated with an increase in mortality (Ihle 2008; Mancini et al 2010).

Novel molecular assays allow for the rapid detection and identification of infection-causing bacteria in a much shorter time-frame. The Prove-it™ sepsis assay, the first microarray-based assay, is capable of identifying up to 50 Gram-positive and Gram-negative species of bacteria which cause the majority of sepsis cases (>90%), as well as identifying mecA, the antibiotic resistance marker used to identify methicillin resistant *Staphylococcus aureus* (MRSA) (see appendix) (Gaibani et al 2009; Mobidiag Ltd 2009). Of the potential sepsis-causing pathogens not detected by the Prove-it™ assay, most account for less than one per cent of all positive blood cultures. However, several clinically relevant pathogens are not detected by the Prove-it™ assay including *Streptococcus viridians*, *Candida* spp and coagulase-negative staphylococci which account for 5.7, 2.0 and 1.1 per cent of all positive blood cultures, respectively (Yoo & Lee 2010). Blood samples are obtained from patients suspected of having sepsis and are cultured using conventional techniques. When samples are confirmed to be positive for sepsis (24-48 hours), DNA is extracted from 0.5ml of the positive culture (Figure 1). The extracted DNA is amplified using PCR and the resulting amplicons are overlaid onto DNA microarrays that are fixed onto the bottom of the reaction tube (TubeArray) or to the bottom of an ELISA plate well (StripArray). Samples are hybridised and stained. The TubeArray allows for a run of

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[1] *The normal white cell count for adults is 4.0-10.0 x 10^9/L.*
[2] Normal $PaCO_2$ is 35-45mm Hg

26 samples whereas the StripArray allows processing of 96 samples. The identification of PCR products is based on the physical position of the positive hybridisation reactions on the array. The samples are read by a fully automated system and a report generated by dedicated software. The assay takes approximately three hours to complete. However, it should be noted that the Prove-it™ assay does not improve *the time to diagnosis* of sepsis but does improve on the *time to positively identify the organism* causing the infection (3hrs vs ≥24 for conventional blood culture), which may be important for the treatment and management of patients (Gaibani et al 2009; Mobidiag Ltd 2009).

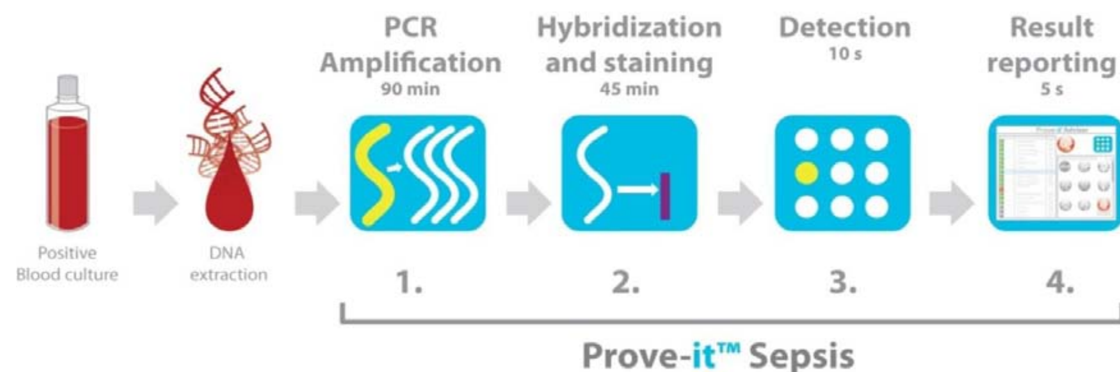

Figure 1 Prove-it™ sepsis assay (Mobidiag Ltd 2009)

Other commercial assays for the identification of sepsis pathogens from positive blood culture samples are the PNA-FISH assay (AdvanDx Inc, USA), a fluorescence in-situ hybridisation assay, and the HyPlex BloodScreen (AmplexBiosystems GmbH, Germany), a multiplex PCR. With the PNA-FISH assay, positive blood cultures are Gram stained and the appropriate assay is selected on the basis of this result. Both assays take approximately three hours to perform but do not detect as many pathogens as the Prove-it™ assay (Mancini et al 2010).

Several PCR-based assays, including the LightCycler® SeptiFast have been developed for the detection of clinically important bacteria involved in sepsis. DNA is extracted and purified from whole blood (3ml) taken from a patient suspected of having sepsis. Three parallel real-time PCR amplification reactions, using the LightCycler® 2.0 system, are performed, one each for Gram-negative and Gram-positive bacteria, and one for fungi. Amplicons are hybridised with specific dye-labelled probes and are continuously detected by automated fluorescence measurement in real time. Amplicons are analysed by dedicated pathogen identification software. The SeptiFast is capable of detecting and identifying 25 different sepsis-causing pathogens (see appendix) in less than six hours (Gaibani et al 2009; Lehmann et al 2008). As with the Prove-it™ assay, *Streptococcus viridians* is not detected by the SeptiFast assay.

Clinical Need and Burden of Disease

There is little documentation on the population incidence of severe sepsis, with most studies reporting on the incidence of sepsis in intensive care units (ICUs). Some

studies have reported that while the incidence of sepsis appears to be increasing, mortality from sepsis is decreasing, however sepsis still remains a major cause of death in ICUs (ARISE et al 2007; Finfer et al 2004).

In 2004 a cohort study documented the incidence of severe sepsis in *adult* ICUs affiliated with the Australian and New Zealand Intensive Care Society. Twenty-three ICUs from 21 Australian and New Zealand hospitals (16 tertiary and 5 university affiliated) participated in the study and reported on all ICU admissions of 48-hours or longer for a 3-month period in 1999 (May-July). During this period there were 5,878 admissions to the 23 ICUs and of these, 2,331 were routine post-operative admissions of less than 48-hours and were therefore not included for analysis, as were the incomplete results from four patients. A total of 3,543 ICU admissions in 3,338 patients (mean age 55.9 ± 20.2 years) were screened for severe sepsis. A total of 691 (20.7%) patients experienced 752 episodes of severe sepsis, equating to 11.8 patients (95% CI [10.9, 12.6]) with severe sepsis per 100 ICU admissions. Of these 691 patients, 183 (26.5%) died in ICU, 224 (32.4%) died within 28-days and 259 (37.5%) in hospital. These data were used to extrapolate the annual incidence of severe sepsis in adult patients admitted to *all* ICUs in Australia and New Zealand, yielding an annual incidence of 0.77 per 1,000 population (Finfer et al 2004).

A later, retrospective study, reported on the outcomes of patients with sepsis admitted to Australian and New Zealand ICUs via the emergency department over a nine year period from 1997 to 2005 (ARISE et al 2007). The number of ICU departments contributing data increased over the study period with 101 ICUs or 82 per cent of all Australian and New Zealand ICUs contributing in the final year. Admission and mortality outcomes for patients are summarised in Table 1.

Table 1 Admission to ICU and outcome data for patients diagnosed with sepsis from emergency

Year	Admissions	Admissions per ICU	ICU mortality %	Hospital mortality %
1997	368	7.7	27.4	35.6
1998	323	6.9	29.1	37.7
1999	291	6.3	22.6	30.7
2000	563	9.1	27.8	25.2
2001	872	11.5	23.3	31.6
2002	1059	11.6	21.7	28.1
2003	1282	13.5	19.7	25.8
2004	1483	14.5	18.5	24.9
2005	1411	14.0	15.6	21.2

The number of patients presenting to emergency with sepsis increased over time, however mortality from sepsis decreased.

In Australia during the year 2007-08, there were 14,643 public hospital separations for sepsis (ICD-10 code A41) and 1,323 (ICD-10 code A40) separations for streptococcal sepsis, representing 142,485 (average length of stay 9.7 days) and

15,740 (average length of stay 11.9 days) patients days, respectively (AIHW 2010). The AIHW also provided data on the number of private hospital separations for the year 2006-07.[3] There were 2,066 and 169 private hospital separations for ICD-10 codes A41 and A40, representing 21,596 and 2,455 patient days, respectively (AIHW 2008).

In New Zealand during the year 2004-05 there were a total of 2,103 public hospital separations for the combined ICD-10 codes A40-41. In 2004, there were 45 patient deaths attributed to sepsis, with 31 and 35 deaths recorded in 2005 and 2006, respectively (Analytical Services, New Zealand Ministry of Health).

DIFFUSION

The Prove-it™ Sepsis product is not currently in use in Australia and New Zealand (personal communication Mobidiag Ltd).
There has been little or no implementation of PCR based diagnostics for bacteraemia in Australia for patients with a clinical diagnosis of sepsis. Many sites have a PCR for meningococcal bacteraemia but this is used in patients with suspected meningococcal disease, many of whom have severe sepsis, but would not be used in patients with severe sepsis in whom there is a low clinical likelihood of meningococcaemia. There are some research based platforms for pneumococcal sepsis, but this would not be offered as a clinical (personal communication ANZICS Clinical Trials Group).

COMPARATORS

Patients suspected of having sepsis are usually diagnosed initially by symptoms, however confirmation of infection by the "gold standard" of blood culture is essential not only to verify infection but also to identify the organisms causing the infection, allowing modification of antibiotic therapy if needed. Samples should be taken from the patients and also from any vascular device in use. Blood culture is performed using continuous monitoring blood culture systems, and, unlike molecular techniques, has the advantage of being able to evaluate the antimicrobial susceptibility of identified pathogens. Guidelines recommend the collection of two to three blood culture sets, each consisting of 20-30ml of blood, per each suspected sepsis episode. Each set is divided evenly for anaerobic and aerobic culture. Microbial growth may be detected in a number of ways: the analysis of CO_2 released; by colorimetric sensors or by monitoring pressure changes in the vessel headspace due to the production or consumption of gases. The sensitivity of blood culture is affected by the volume of blood cultured, with poor results obtained from samples with inadequate volume. More reliable results are obtained when the time from sampling to culture is reduced to a minimum. In addition, some bacteria are slow growing or difficult to culture. A positive signal from culture may be obtained within 24-48 hours. A Gram stain is then

[3] Public hospital separation data from this same period would have been supplied however the number of separations differed markedly from those in the National Morbidity Database (ICD-10 code A41: 11,403 vs 13,469 separations)

performed directly on the cultured blood sample and other biochemical tests are performed to identify the pathogen which may include 16S rDNA PCR[4]. In addition, tests for susceptibility to antimicrobial agents are performed. Identification of pathogens takes approximately 18-24 hours, however this time may be markedly increased for slow growing pathogens. Total time for a positive diagnosis and identification of pathogens may therefore take a minimum of 48 hours to 72 hours or more, depending on the pathogen (Mancini et al 2010).

The MBS item number, 69354 is the blood culture for pathogenic micro-organisms (other than viruses), including sub-cultures and (if performed): (a) identification of any cultured pathogen; and (b) necessary antibiotic susceptibility testing; to a maximum of 3 sets of cultures - 1 set of cultures (Fee: $30.95). In 2009, there were 140,206 services provided for the item number 69354.

SAFETY AND EFFECTIVENESS ISSUES

Only two studies were identified which reported on the use of the Prove-it™ assay. The first study was reported in an abstract presented to the European Congress of Clinical Microbiology and Infectious Diseases in 2009 (Allais et al 2009). Fifty-nine clinical samples were selected after blood culture and 16S rDNA PCR had identified the pathogens responsible for the infection. Samples were culture positive (C^+, n=10), culture negative but PCR positive (C^-/ PCR^+, n=49) or negative by both methods (C^-/ PCR^-, n=10). Samples were selected on the basis that the infective pathogens were included in the spectrum able to be detected by the microarray, and therefore the sample population is affected by spectrum bias[5] (level III-3 diagnostic evidence). All of the C^-/ PCR^- were negative by the Prove-it™ assay, however only 7/10 (70%) of the C^+ and 34/49 (69.4%) of the C^-/ PCR^+ were positive by the Prove-it™ assay. Although the authors felt that these preliminary results were promising, it would appear from these results that the Prove-it™ assay is not ready for use in a clinical situation where it would be used to identify pathogens for treatment management purposes.

A more clinically relevant, large cross-classification study conducted in Finland reported on the results of 3,318 blood samples obtained from patients with suspected sepsis (level III-1 diagnostic evidence). All samples underwent blood culture followed by the Prove-it™ assay only if the blood culture results were positive. 16S rDNA PCR was performed on discordant results (Tissari et al 2010).

There were 2,107 (63.5%) positive results by culture, and of these 1,807 (85.8%) were found to be positive by the Prove-it™ assay, indicating that 14 per cent of positive

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[4] The 16S rDNA sequence is a gene encoding small subunit ribosomal RNA. This gene contains conserved sequences of DNA common to all bacteria and divergent sequences unique to each species of bacteria

[5] Spectrum bias refers to the evaluation of a diagnostic test in a biased group of patients which leads to an overestimation of the sensitivity and specificity of the test.

sepsis samples were caused by bacteria *unable* to be classified by the Prove-it™ assay Of those samples positive by blood culture, 664 were a Gram-positive and 1066 were a Gram-negative bacteria species covered by the pathogen panel of the Prove-it™ assay. The Prove-it™ assay detected 645 of the 664 Gram-negative samples, a concordance of 97.1 per cent for those bacteria covered by the Prove-it™ panel. Similarly there was a concordance of 96.7 per cent for the Gram-positive bacteria covered by the Prove-it™ panel. Fifty-two samples were considered to be false positives, with 18 of these considered true false positives and the remaining 34 attributed to contamination or sampling errors. These samples were removed from the final analysis to give a specificity of 98.8 per cent (95% CI [98.1, 99.2]), however if these samples were included in the analysis, the specificity still would be excellent at 96.6 per cent. Sensitivity of the Prove-it™ assay was 94.7 per cent (95% CI [93.6, 95.7]). Ninety-four samples were regarded as false negatives (5.3%) (Table 2).[6]

Table 2 Accuracy of the Prove-it™ sepsis assay

		Disease		
		+ve	-ve	
Test	+ve	1696	18 (52)	1714 (1748)
	-ve	94	1476	1590
		1790	1494 (1528)	3284 (3318)

Culture results identified 1,670 samples which were infected by bacteria that were covered by the Prove-it™ panel, 304 samples which were not covered and 133 samples which were infected by more than one bacteria covered by the panel. The Prove-it™ assay had difficulty resolving more than half of these polymicrobial samples, with 60 of these samples being noted as false negatives and 11 as false positives.

Turnaround assay times were noted for a small subset of samples (n=39), with the median time difference between the Prove-it™ assay and conventional culture of 18 hours and 19 minutes and a range of 17 hours, 29 minutes to 43 hours and 8 minutes (Figure 1). Being able to identify the bacterial species responsible for the sepsis-causing infection 18 hours earlier may be of clinical importance in the management of some patients.

[6] Pathogens not covered by the Prove-it™ assay but detected by blood culture: 341 specimens, some of which were mono-microbial and some poly-microbial expressed as a % of the total blood culture positive samples (2107): *Streptococcus viridans* (5.7%), *Candida* spp (2.0%), Coagulase-negative staphylococci (1.1%), Diphtheroid (0.9%), *Propionibacterium* sp (0.9%), *Bacillus* sp (0.7%), *Micrococcus* sp (0.6%), *Aerococcus* sp (0.5%), *Enterococcus* sp (0.4%), *Clostridium* sp (0.3%), *Fusobacterium* sp (0.2%), *Pseudomonas* sp (0.2%), *Capnocytophaga* sp (0.2%), Vibrio sp (0.2%), *Pasteurella* sp (0.1%), *Campylobacter* sp (0.1%), *Neisseria* sp (0.1%), others (2.0%)

	Blood culture	Identification	Turn-around time
Standard assay	15 hours	2.5-9 hours	41.5-48 hours
Prove-it™ assay	15 hours	8 hours	23 hours

Figure 2 Turnaround time for conventional culture vs Prove-it™ assay (adapted from Lin & Yang 2010)

The Prove-it™ assay should not be used as a replacement for conventional blood culture, however it is a useful adjunct in the determination of sepsis-causing infections.

Several papers were identified that reported on the use of the LightCycler® SeptiFast assay to diagnose sepsis, however due to the limitations of the size of this summary, only the results from two of the most recent and largest studies will be summarised.

A large cross-comparative study reported on the ability of the SeptiFast assay to diagnose patients (n=306) admitted to the emergency department with suspected sepsis, compared to conventional blood culture (level III-1 diagnostic evidence). Blood samples were taken and conventional blood culture and PCR were conducted. Patient records were independently examined by two clinicians to determine whether or not a patient could be considered as ever having sepsis, based on discharge status, likelihood of infection, causative organism and patient outcome. A diagnosis of sepsis using these criteria infection was made in 263 (86%) patients.

Using the above clinical criteria as a diagnosis, blood culture alone was positive in 66/263 (25.1%) of cases but correctly identified all negative cases (specificity 100%), as did PCR alone. PCR alone identified only 53/263 (20.2%) and PCR combined with blood culture identified 79 (30%) of positive clinical criteria cases. When blood culture was used as the reference standard, PCR had a sensitivity and specificity of 60.6 and 94.6 per cent, respectively. The sensitivity would be considered low, with a false negative rate of 39.4 per cent, indicating that if using PCR alone to diagnose sepsis, a high proportion of patients would not be treated appropriately. The positive and negative predictive values were 75.5 and 89.7 per cent, respectively. The kappa statistic for the agreement of blood culture and PCR was moderate at 0.59[7] (95%CI [0.48, 0.69]) (Tsalik et al 2010).

The study by Lehmann et al (2009) did not report on the diagnostic accuracy of the SeptiFast compared to conventional blood culture, but rather reported on the change on patient management (changes to antimicrobial therapy) when the results of SeptiFast, in conjunction with the results of blood culture, were used (level III-2 intervention evidence). Consecutive patients (n=436) with 467 episodes of suspected sepsis were enrolled. Blood samples were taken and conventional blood culture and PCR were conducted.

[7] A kappa statistic of 1 indicates perfect agreement and a kappa statistic of zero indicates agreement equivalent to chance.

Blood culture identified 117 clinically relevant bacterial infections from 99 episodes and PCR identified 154 bacteria involved in 131 episodes, 22 of which were polymicrobial. Antimicrobial treatment was altered in 135 of the 467 episodes depending on the results of blood culture or PCR. A change in therapy occurred in 49 episodes based on a positive blood culture result. There was a total of 127.8 days, or an average of 2.6 days per episode, spent on inadequate antimicrobial therapy. A change in therapy occurred in 46 episodes based on a positive PCR result, with a total of 106.5 days, or 2.3 days per episode, spent on inadequate antimicrobial therapy. In 24 of these episodes, the results of PCR preceded those of blood culture by an average of two days and in 22 episodes PCR was more sensitive than blood culture, with the potential to reduce inadequate antimicrobial therapy by an average of 2.6 days per episode. Of the PCR positive episodes, 85/131 (64.9%) were receiving appropriate antimicrobial therapy and no change was made to therapy once PCR results were made known. Treatment was changed in nine of these 85 episodes before the PCR results were known, based on the result of Gram negative staining. It was also estimated that using the PCR results may result in the *over treatment* of patients in 18 of these episodes.

An average of 22.8 days on early adequate antimicrobial treatment could be gained per 100 PCR tests conducted. A subgroup analysis revealed that this effect was higher in episodes from patients in intensive care or a surgical ward (36.4 gainable days, p=0.002) compared to emergency or other wards (10.6 gainable days). A covariate analysis revealed that PCR was of greater benefit in patients with an underlying diagnosis of invasive disease[8] (327 episodes) compared to other diagnoses, with an adjusted odds ratio of 3.9 (95% CI [1.4, 11.3]). PCR was also of greater benefit in episodes where the patients were aged ≥56 years compared with younger patients, with an adjusted odds ratio of 2.8 (95% CI [1.4, 5.9]). These results indicate that the use of PCR in conjunction with conventional blood culture may be useful for the early identification of sepsis causing bacteria, and may play an important role in the change of patient management resulting in better patient outcomes.

COST IMPACT

Although the Prove-it™ Sepsis assay is not currently available in Australia, the company Mobidiag Ltd provided current prices for their product in Europe. The Prove-it™ Sepsis TubeArray System, which includes a laptop, software, the TubeArray reader and a data matrix reader is currently marketed at €10,000. This system can be used with other Prove-it™ applications including a herpes assay. The Prove-it™ Sepsis assay kit which includes enough reagents for 25 assays costs approximately €1,400. The Prove-it™ Sepsis Strip Array System costs approximately €16,000 and includes instrumentation for result analysis: the StripArray reader with

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[8] intra-abdominal sepsis, nosocomial pneumonia, catheter-related sepsis, multi-organ dysfunction, neutropenic fever and pyelonephritis

an embedded computer and software. The system is also a onetime investment and can be used with other Prove-it™ products/applications including a Candida infection assay. The Prove-it™ Sepsis Strip Array kit, which includes enough reagents for 48 assay runs, will cost approximately €2,100, depending on sales volumes (personal communication Mobidiag Ltd).

Roche were contacted regarding the price of the LightCycler system, however no response was received.

ETHICAL, CULTURAL OR RELIGIOUS CONSIDERATIONS

No issues were identified/raised in the sources examined.

OTHER ISSUES

Five of the 14 authors of the Tissari et al (2010) paper describing the use of the Prove-it™ assay were employees of Mobidiag, the manufacturers of the assay.

SUMMARY OF FINDINGS

The Prove-it™ assay provides rapid identification of a large number of bacterial species involved in sepsis infection once sepsis has been diagnosed by conventional means. Appropriate changes in patient antimicrobial therapy may take place 18 or more hours earlier than with blood culture, however the number of false negatives and false positives make this technology suitable as only an adjunct to blood culture. Severely ill patients may still be better served waiting for blood culture results, especially as the Prove-it™ assay does not provide any information on the antimicrobial susceptibility of detected pathogens. The SeptiFast assay has pros and cons associated with it as well. On the plus side, the SeptiFast can be used on whole blood while not waiting for the results of blood culture, however the number of pathogens able to be detected by the SeptiFast is reduced in comparison to the Prove-it™ assay. The reported specificity of the SeptiFast assay is high, indicating that it is correctly identifying those patients who do not have sepsis and may be receiving antimicrobial treatment unnecessarily. However, the reasonably low sensitivity indicates that the assay is poor at correctly identifying those patients who do have sepsis, and therefore many patients may not receive antimicrobial treatment if treatment was based on the PCR test alone. Both assays appear to be of value if used in conjunction with blood culture as time to correct therapy is vitally important in the treatment of sepsis.

HEALTHPACT ASSESSMENT:

There is a large body of evidence describing the use of rapid molecular tests for either the diagnosis of sepsis or the rapid identification of bacteria involved in sepsis infection. As sepsis is associated with high levels of mortality, there is a clear clinical need to identify the causative agents involved in the infection as rapidly as possible. Rapid molecular tests, used in conjunction with conventional blood culture, may

result in changes to patient management, which may be reflected in improved patient outcomes in terms of both mortality and morbidity. HealthPACT action is pending on the determination of whether sufficient evidence exists for conducting a Horizon Scanning Report.

NUMBER OF INCLUDED STUDIES

Total number of studies	4
Level III-1 diagnostic evidence	2
Level III-3 diagnostic evidence	1
Level III-2 intervention evidence	1

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Search Criteria to be used:

Sepsis/*diagnosis
Bacteremia/microbiology*

Bacteriological Techniques

DNA, Bacterial/analysis*

Nucleic Acid Amplification Techniques

Oligonucleotide Array Sequence Analysis*

Polymerase Chain Reaction*

APPENDIX

Pathogens detected by Prove-it™ (Mobidiag Ltd 2009)

Gram+	Gram-
Identified targets	Identified targets
mecA methicillin resistance marker	*Acinetobacter baumannii*
	Enterobacter aerogenes
Clostridium perfringens	*Enterobacter cloacae*
Enterococcus faecalis	*Escherichia coli*
Enterococcus faecium	*Haemophilus influenzae*
Listeria monocytogenes	*Kingella kingae**
*Propionibacterium acnes**	*Klebsiella oxytoca*
Staphylococcus aureus	*Klebsiella pneumoniae*
Staphylococcus epidermidis	*Neisseria meningitidis*
Streptococcus agalactiae	*Proteus mirabilis*
Streptococcus dysgalactiae subspecies *equisimilis*	*Proteus vulgaris*
Streptococcus pneumoniae	*Pseudomonas aeruginosa*
Streptococcus pyogenes	*Salmonella enterica* subspecies *enterica*
	Serratia marcescens
	Stenotrophomonas maltophilia
Coagulase negative *Staphylococcus*	
	Bacteroides fragilis group
	Campylobacter jejuni/coli
	Enterobacteriaceae
	Neisseria sp. non-*meningitidis*

Bacteroides fragilis detects at least the following species: *B. fragilis, B. vulgatus, B. thetaiotaomicron.*

Coagulase negative *Staphylococcus* detects at least the following species:
S. haemolyticus, S. hominis S. lugdunensis, S. saprophyticus, S. warneri, S. xylosus.

Enterobacteriaceae detects at least the following species: *Citrobacter amalonaticus, Citrobacter braakii, Citrobacter freundii, Citrobacter koseri, Enterobacter hormaechei, Enterobacter sakazakii, Kluyvera intermedia, Morganella morganii, Pantoea agglomerans, Providencia rettgeri, Providencia stuartii, Yersinia enterocolitica, Yersinia pseudotuberculosis.*

Neisseria sp., non-*meningitidis* covers at least the following species: *N. gonorrhoeae, N. subflava, N. sicca, N. cinerea, N. elongata* subspecies *nitroreducens, N. flavescens, N. lactamica, N. zoodegmatis.*

Salmonella enterica subspecies *enterica* detects at least the following serovars: Enteritidis, Oranienburg, Othmarschen, Panama, Paratyphi, Stanley, Typhi, Typhimurium, Virchow, group A,B,C,D.

*Available only on Prove-it™ StripArray platform.

Pathogens detected by SeptiFast (Roche Diagnostics 2010)

Gram (-)	Gram (+)	Fungi
• *Escherichia coli* • *Klebsiella (pneumoniae / oxytoca)* • *Serratia marcescens* • *Enterobacter (cloacae / aerogenes)* • *Proteus mirabilis* • *Pseudomonas aeruginosa* • *Acinetobacter baumannii* • *Stenotrophomonas maltophilia*	• *Staphylococcus aureus* • CoNS (Coagulase negative *Staphylococci)** • *Streptococcus pneumoniae* • *Streptococcus spp.*** • *Enterococcus faecium* • *Enterococcus faecalis*	• *Candida albicans* • *Candida tropicalis* • *Candida parapsilosis* • *Candida krusei* • *Candida glabrata* • *Aspergillus fumigatus*

*S. epidermidis, S. haemolyticus **S. pyogenes, S. agalactiae, S. mitis