Fungitell® is a new test offered to detect the presence of certain invasive fungi in the blood. It indicates the presence of these fungi well before they are cultured in clinical specimens, and thus has utility in the early detection of invasive fungal infections.

*Candida* species are now the 4th most commonly isolated organism in nosocomial bloodstream infections. They are associated with significant morbidity and mortality; attributable mortality has been shown to be as high as 47%. Invasive candidiasis has a non-specific presentation; clinical and radiological signs appear late in the course of the disease. On the other side of the coin it is difficult to distinguish colonisation from invasive disease in high-risk patients. This inability to identify infected patients early in the course of the disease contributes in no small way to the significant mortality.

Blood cultures are positive in only 50% of cases of invasive candidiasis (some studies have suggested less than 30% of cases), unfortunately occurring in more advanced disease, when the fungal burden is higher. Less than 10% of invasive aspergillosis cases have positive blood cultures. The use of prophylactic fluconazole has decreased the sensitivity of blood cultures even further. Less than 50% of broncho-alveolar lavage specimens are positive in patients with invasive pulmonary aspergillosis.

The gold standard has remained the histopathological demonstration of organisms in tissue or the growth of fungi from normally sterile body fluids. This is, however, difficult to apply in the clinical setting.

Timing of therapy has a significant impact on mortality; patients in whom antifungal therapy is started within 12 hours of drawing fungal blood cultures have a 20% lower mortality than patients in whom therapy is delayed for 24 hours. *Candida* spp generally take at least 18 to 72 hours to grow in conventional blood cultures, mostly outside the treatment window. Hence the early identification of patients requiring antifungal therapy assumes great importance. There is a clear need for laboratory investigations which indicate such critical patients early on; it is intuitive that this confers a survival benefit.

Non-culture-based laboratory methods of detecting fungal infections have become increasingly relevant in managing at-risk patients. New methods of detecting invasive candidiasis include:

1) polymerase chain reaction (PCR), not available in South Africa yet
2) CAGT – *Candida albicans* germ tube antibodies, also not available in South Africa
3) GM – galactomannan, cell wall antigen in *Aspergillus* spp
4) 1,3 beta-D (B-D) glucan

GM and 1,3 B-D glucan are so-called ‘surrogate markers’.

1,3 B-D glucan is a cell-wall component of most medically important fungi, including:
- *Candida* spp
- *Aspergillus* spp
- *Fusarium* spp
- *Acremonium* spp
- *Pneumocystis jirovecii*
- *Sporothrix schenckii*
- *Coccidioides immitis*
- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- *Trichosporon* spp
- *Saccharomyces cerevisiae*

1,3 B-D glucan is NOT found in:
- *Cryptococcus* spp
- *Zygomycetes* (Adsidia, *Mucor*, *Rhizopus*)

Lower levels of 1,3 B-D glucan are found in the cell wall of *Candida parapsilosis*, which may comprise more than 30% of *Candida* isolates in neonatal intensive care units. This, together with the fact that baseline levels in uninfected children are, as yet, undetermined, diminishes the tests’ utility in the paediatric population. Higher levels than in uninfected adults have been reported; work is currently underway to determine these critical cutoff values. *C. parapsilosis* may also infect adults with chronic indwelling intravenous catheters.

1,3 B-D glucan is a qualitative test; values are reported as follows:
- <60 pg/mL: NEGATIVE
- ≥80 pg/mL: POSITIVE (in an at-risk patient)
- 60-79 pg/mL: EQUIVOCAL; additional sampling is necessary

A positive result does NOT indicate which species is present, and should be used in conjunction with clinical findings.

The specificity of the test is from 87% to 90% on a single positive result. Specificity increases to greater than 96% for two or more sequential positive results. Sensitivity varies between 64% to 70%.
False positives may be found under the following circumstances:
- bacteraemic patients
- haemolysed specimens
- excess manipulation of samples (glucan contamination)
- antibiotics; only amoxicillin/clavulanate and piperacillin/tazobactam (of note, antifungal therapy does not significantly affect the performance of the assay).
- haemodialysis with cellulose membranes
- patients treated with ivi products manufactured using cellulose filters, eg.
  - human immunoglobulins
  - albumin
  - coagulation factors
  - plasma protein fraction
  - anti-tumour polysaccharides
- certain surgical gauzes/sponges – transient false positives in surgical patients
- heel-sampling in neonates

False negatives may be seen in the following circumstances:
- immune complex formation
- Candida parapsilosis infections, as discussed above

False positives tend to rise and fall suddenly; a more protracted rise is indicative of true invasive infection. It has been suggested that surrogate markers should be used in combination to minimise the deficiencies of each; in doing so, the specificity of each test is significantly increased.

GM is a cell wall antigen in Aspergillus spp. This surrogate marker is available as the Platelia Aspergillus test. It is a highly specific test, although sensitivity has been reported to vary between 30% and 100%. Up to 83% false positives have been detected in neonates which precludes its use in this population group. False positives have also been described in patients receiving beta-lactam therapy, while antifungals tend to lower the sensitivity significantly. There is a tendency to peak later than 1.3 B-D glucan. Sensitivity in liver transplant patients is 50%, in lung transplant patients 30%.

Who to use the Fungitell test in?
A pre-emptive approach is recommended, whereby surveillance is carried out in at-risk patients. Aspergillus infections are seen largely in neutropenic patients and patients with haematological malignancies especially acute myeloid leukaemia and myelodysplastic syndrome. Twice weekly testing has been recommended as a screening strategy; both in invasive aspergillosis as well as invasive candidiasis, this increases the sensitivity, specificity as well as the positive predictive value (PPV) of the assay.

Invasive candida infections are seen in a more heterogeneous group, particularly in critically ill patients, in whom the most important risk factor is length of stay in intensive care, the peak incidence being on day 10. Of note, colonisation increases dramatically after day 8 in an intensive care unit (ICU). The following are commonly accepted risk factors for invasive candidiasis:

Adults:
- prolonged length of stay in ICU
- high Acute Physiology and Chronic Health Evaluation II (APACHE) score (eg. >20)
- renal failure
- haemodialysis
- broad-spectrum antibiotics
- central venous catheter
- parenteral nutrition
- immunosuppressive drugs
- uncontrolled hyperglycaemia
- cancer and chemotherapy
- severe acute pancreatitis
- candida colonisation at multiple sites
- surgery
- transplantation

Neonates and children:
In addition to the adult risk factors:
- prematurity
- low APGAR score
- congenital malformations

Predicting therapeutic outcome of patients on anti-fungal therapy (i.e. demonstrating falling levels of 1.3 B-D glucan) is another area where 1,3 B-D glucan testing is of great use. This has so far only been demonstrated, however, with invasive aspergillosis, where decreasing levels of 1,3 B-D glucan have been shown in patients who recovered from invasive aspergillosis. It is felt that increasing, or persistently high levels of 1,3 B-D glucan in patients on antifungal therapy is likely to indicate a poorer prognosis.

In conclusion, this is an assay whose major utility lies in the diagnosis of invasive fungal infections before clinical or microbiological diagnosis. It is, however, not a replacement for careful clinical and microbiological examination.

References
6. Patterson, TF. Infectious Disease Clinics of North America, September 2006; 28(3)