Objectives: Sepsis is an important cause of morbidity and mortality. Rapid identification of bloodstream pathogens is essential for correct antimicrobial treatment and to achieve better patient outcome. The aim of this study was to evaluate the usefulness of multiplex PCR (Magicplex™ Sepsis Test, Seegene) (MP) for the diagnosis of bloodstream infections. This test allows detection of more than 90 sepsis-causing pathogens within 6h. Methods: Blood samples from patients with suspected sepsis were collected. Four blood culture (BC) bottles and one EDTA tube with 1 ml of whole blood for PCR from each patient were processed. The extraction was performed with the Seegene Blood Pathogen Kit and Seeprep12™ extractor. Amplification, screening and identification were performed following the manufacturer’s instructions. Blood cultures were processed and incubated with Bactec-9240® (Becton Dickinson). Blood samples not processed within 24h after collection and inhibited samples were excluded from the study. In 34 samples pathogens detected by MP were identified only to the genus level (Staphylococcus spp., Streptococcus spp.) or bacteria group for Gram-negative bacilli. These samples were excluded from the data analysis. Results: Valid results were obtained for 778 processed blood samples by MP, BC or both methods. Excluding contaminants, 131/701 (18.68%) samples were positive: 29/131 (22.13%) were detected by both methods, 35/131 (26.71%) only by MP and 67/131 (51.14%) only by BC. The rate of positive BC was 96/701 (13.69%) whereas that of positive MP was 64/701 (9.12%). Considering BC to be the “gold standard” method, MP demonstrated a sensitivity of 30.2%, a specificity of 94.21%, a positive predictive value of 45.31% and a negative predictive value of 89.48%. MP detected 35 additional positive samples that BC failed to detect (26.71%). Conclusions: Although the sensitivity of MP is low, multiplex PCR combined with blood culture improves the detection of bloodstream pathogens, especially in patients from intensive care units and with previous antibiotic therapy. Blood culture remains the gold standard for the diagnosis of bloodstream infections.