Karius Inc.
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KARIUS™

Multi-pathogen detection from a single blood draw

Karius has developed a novel commercial cell-free DNA blood test able to rapidly and accurately detect more than 1,000 pathogens from a standard blood draw.

The discovery that blood carries short fragments of DNA, known as cell-free DNA (cfDNA), has opened a new window into biological processes and, in combination with advances in sequencing, is transforming diagnostics. Up until now, technical challenges have precluded the application of cfDNA to infectious disease. Karius has addressed these hurdles to develop a commercial test that allows rapid, accurate detection of more than 1,000 pathogens from a standard blood draw.

The Karius Test has broad applications in clinical care, drug discovery, and research, offering "a non-invasive assay for identifying pathogens causing disease throughout the body," according to CSO Tim Blauwkamp. Because the Karius Test can quickly detect pathogens and monitor infections without the need for biopsy or the growth of organisms in culture, it has the potential to transform clinical practice.

The initial inspiration for the Karius Test was the discovery of pathogen cfDNA in blood samples from patients with fever who had undergone organ transplantation. For such patients, effective treatment requires rejection to be distinguished from infection as the cause of the fever. With standard microbiological testing, this may require the biopsy of an already fragile patient and a wait for culture results that ultimately may prove inconclusive. A cfDNA blood test has clear advantages for both the patient and the physician, as a noninvasive alternative to biopsy that sensitively detects infection by direct sequencing. These benefits are equally relevant in other clinical scenarios, including sepsis, bloodstream infections (BSIs), deep-tissue infections, febrile neutropenia, and in other immunocompromised patients.

According to CEO Mickey Kertesz, "The Karius Test arms physicians with a single test that can deliver a potentially life-saving result, often more quickly than traditional testing methods, when time is critical." The Karius pathogen test is engineered to achieve the rigor and speed needed for routine clinical use. It is a rapid cfDNA test, in most cases with a turnaround time of 1–2 days from sample receipt. The Karius Test requires no special blood collection tube or sample preparation; plasma preparation tubes can be used, and samples are shipped to Karius at ambient temperature for processing in their Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists (CAP)-accredited laboratory.

As shown in Figure 1, once the sample is received and accessioned at Karius, the cfDNA is isolated and a sequencing library is prepared. Next, the amplified, tagged sample is sequenced and analyzed overnight, and in most cases the next day a report is generated identifying any pathogens detected.

Fig. 1 | The Karius Test. The test rapidly detects more than 1,000 clinically relevant microbes by sequencing and analyzing pathogen-derived cell-free DNA (cfDNA) isolated from a standard blood draw or plasma sample. PPT, plasma preparation tube.

Because pathogen cfDNA represents only a small fraction of total circulating cfDNA, achieving a good signal-to-noise ratio was critical to the development of a robust test. The Karius team has developed custom methods to:

- remove human cfDNA, which represents >99% of total circulating cfDNA;
- minimize environmental nucleic acid contamination and identify carry-over from sample to sample or batch to batch;
- address incorrect sequence attribution by compiling and curating a unique sequence database and developing algorithms that account for the diversity of 'wild' pathogens seen clinically;
- achieve a broad dynamic range to quantify levels of pathogen cfDNA that can vary widely from patient to patient.

The real-world utility of the Karius Test has been demonstrated in sepsis, BSI, febrile neutropenia and others. A study (SEP-SEQ) conducted at the Stanford University Hospital Emergency Department, which enrolled 350 patients with suspected sepsis, compared Karius testing with standard methods, and found that the Karius Test identified a potential sepsis-causing pathogen in three times as many patient samples as blood culture (67.7% versus 18.1%, respectively) and more frequently than all conventional microbiological methods combined (57.5%)[1]. In this study, utilizing post-hoc clinical adjudication of all test results, the Karius Test had a sensitivity of 93.9% and a specificity of 76.3% when compared with blood culture[1]. Similarly, in a separate study of patients with febrile neutropenia, the Karius Test proved a more sensitive method for the detection of pathogens, capturing a signal in some samples with negative cultures[2]. In a third study, the Karius Test detected the continued presence of pathogen cfDNA in patients for a duration of 6.5 days, versus 3.4 days for blood cultures, in patients with BSI undergoing treatment[3]. Together, the results show that incorporating Karius testing into clinical practice has the potential to deliver rapid, sensitive results, significantly improving on the current standard of care in many clinical cases.

Karius is proving it can add value during drug development as well. For example, Nohla Therapeutics is using the Karius Test to detect subclinical infection in a phase 2 study of acute myeloid leukemia patients with chemotherapy-induced neutropenia. "Patients undergoing intensive chemotherapy have a high risk of infectious complications, which often are not detected by standard microbiologic techniques," said Karius vice president of medical affairs and clinical development, David Hong. "Our test allows quantitative monitoring of pathogen cfDNA to help assess patients receiving novel therapies." Karius is also reaching out to research institutions, offering two clinical investigator awards of up to $50,000 each, to accelerate the discovery of new applications for the Karius Test that may positively impact human health. "Our goal is to work collaboratively with industry and academic partners to collectively drive the field forward," said Blauwkamp.

1. Thair, S. A. et al. The SEP-SEQ trial: clinical validation of the Karius plasma next-generation sequencing test for pathogen detection in sepsis. *Open Forum Infect. Dis.* **4**, S735 (2017).
2. Benamu, E. et al. Performance of the Karius plasma next generation sequencing test in determining the etiologic diagnosis of febrile neutropenia: results from a pilot study. *Open Forum Infect. Dis.* **4**, S613–S614 (2017).
3. Wanda, L. et al. Direct detection and quantification of bacterial cell-free DNA in patients with bloodstream infection (BSI) using the Karius plasma next generation sequencing (NGS) test. *Open Forum Infect. Dis.* **4**, S613 (2017).

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