Abstract

Having fever is a common symptom during the course of infectious diseases. In the early stage of the disease development, it is often difficult to detect the causing agent by the conventional culture method, especially in the case of bacteremia before sepsis and fever is the only sign before the acute syndrome to show up. During this period, the concentration of the responsible pathogen is usually too low to be detected and thus fails in getting in time the needed accurate diagnosis and treatment.

The success rate of blood culture is less than 20%. Therefore, we may have to rely on molecular diagnoses such as bacterial DNA detection and PCR methods in such circumstances because such approaches are fast and require small sample amount.

In this study, we used 16S rRNA as the target gene. It is one of the operon genes of ribosomal DNA present in all bacteria. The gene is very stable and changes only in accordance with the evolution of the bacterium, so it is a good indicator for differentiating between bacterium species. The entire length of 16S rRNA is about 1600bp. Recently, it has been found that in its anterior part of 350bp there are three specific regions, which sequential variations are enough to reflect the differences among all bacterium species ever reported. With direct comparison to database, one can easily identify all bacteria under investigation.

Our whole blood samples come from hospital emergency wards and most of which are collected from patients in the early stage of infections. Therefore, successful early diagnosis is essential to intervene the disease development.

Our standard detection procedure includes performances of PCR and RT-PCR as well as blood culture isolation for comparison purposes. In our method development, we first tested PCR and found although it worked fine with artificial samples, it fell short when dealing with real clinical samples. We assume the reason would be either the amount of bacterium was too low for the method or there were some unknown inhibiting factors existing in the blood. However, when we used RT-PCR to assay m-RNA, the result we got is quite satisfactory. In two test cases, we successfully detected *S. pneumoniae* as the causing agent while the culture method turned out nothing but negative results.

Recently, a new device for identification of pathogens using 16S rRNA as the target gene has become available on the market, which sounds similar to the idea in our study. However, the sample required for the kit to diagnose is said having to be culture isolation. Now, it posts an obvious problem, i.e. how are we going to apply this new kit to identify the causing agent if we could not isolate any bacterium particles at the first place? It seems somewhat similar to the problem we came across with the PCR trial when we have difficulty to detect the existence of bacteria in early stage due to too low a concentration. After all, our study shows RT-PCR is a good method for such circumstances, except its stability may be a problem for us to study in the future.

Keywords：DNA；PCR reaction；Primer；Probe