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Kemampuan PCR Gen psaA untuk Mendeteksi Inokulum Streptococcus pneumoniae dalam Media Cair = The Performance of psaA Gene PCR to Detect Streptococcus pneumoniae in Inoculated Liquid Media

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Abstrak

[Deteksi Streptococcus pneumoniae (pneumokokus) dilakukan dengan metode biakan dan PCR. Tujuan penelitian menentukan batas kemampuan tehnik PCR gen psaA mendeteksi inokulum pneumokokus dalam media cair sebelum inkubasi dan setelah inkubasi 24 jam. Penelitian secara eksperimental menggunakan S.pneumoniae ATCC (American Type Culture Collection) 49619 yang ditumbuhkan pada media agar darah domba. Sepuluh mililiter suspensi bakteri dengan densitas 6x107/ml, 6x106/ml, 6x105/ml, 6x104/ml, 6x103/ml, 6x102/ml, 60/ml, 6/ml dimasukkan dalam media cair BD BACTEC™ Plus Aerobic/F Culture Vials. Masing-masing densitas diinokulasikan ke dalam 20 media cair tersebut. Selanjutnya, dari tiap media cair yang telah diinokulasi, sebelum inkubasi maupun setelah inkubasi 24 jam, dilakukan pewarnaan Gram, diinokulasikan pada media agar darah domba, serta uji PCR untuk mendeteksi gen psaA. Bila ditemukan pertumbuhan koloni pneumokokus pada media agar darah, dilanjutkan uji katalase dan sensitivitas optochin. Uji PCR psaA ”positif” bila ditemukan amplikon dengan berat molekul 838 pasang basa. Metode biakan dan PCR dinyatakan“mampu mendeteksi pneumokokus” bila > 60% dari 20 replicate memberikan hasil positif. Dari masing-masing 20 replicate dengan densitas bakteri dalam inokulum awal 6x107/ml, 6x106/ml, 6x105/ml, 6x104/ml, 6x103/ml, 6x102/ml, 60/ml, 6/ml sebelum inkubasi, jumlah replicate yang terdeteksi gen psaA berturut-turut adalah 9/20 replicate (45%), 9/20 (45%), 3/20 (15%), 1/20 (5%), 0/20 (0%), 0/20 (0%), 0/20 (0%), 0/20 (0%). Setelah inkubasi 24 jam berturut-turut adalah 20/20 replicate (100%), 18/20 (90%), 11/20 (55%), 8/20 (40%), 4/20 (20%), 2/20 (10%), 0/20 (0%), 0/20 (0%). Dari data kadar DNA ekstrak terlihat uji PCR psaA penelitian ini membutuhkan kadar DNA 84 ng/µL. Hasil penelitian menunjukkan diperlukan inkubasi 24 jam agar terdeteksi oleh uji PCR psaA dengan densitas pneumokokus dalam inokulum awal minimal 6x106/ml. Kelemahan penelitian adalah proses ekstraksi DNA tidak optimal sehingga kadar DNA ekstrak sangat bervariasi dan menyebabkan gen psaA tidak terdeteksi sebelum inkubasi.;Streptococcus pneumoniae (pneumococcal) detection can be done by culture and PCR methods. The purpose of this study was to determine the limits of psaA gene PCR in detecting pneumococcal inoculum prior to incubation and after 24 hours of incubation of liquid media. This experimental study used Streptococcus pneumoniae ATCC (American Type Culture Collection) 49619 which was grown on sheep blood agar. Ten mililiter of bacterial suspensions with initial density of 6x107/ml, 6x106/ml, 6x105/ml, 6x104/ml, 6x103/ml, 6x102/ml, 60/ml, 6/ml were inoculated into liquid media, BD BACTEC™ Plus Aerobic/F Culture Vials. Each bacterial density was inoculated into these 20 liquid medias. From each inoculated BD BACTEC™ Plus Aerobic/F Culture Vial, prior to incubation and after 24 hours of incubation, Gram staining, subculturing on sheep blood agar, and psaA gene PCR were done. When pneumococcal colonies were found on sheep blood agar, the colonies were tested for catalase and optochin sensitivity. PsaA gene were determined as “positive” when amplicons with molecular weight 838 pairs of bases were found. Culture and PCR methods were determined as able to detect pneumococcus when > 60% of 20 replicates yield positive results. The psaA PCR positive result rate of initial bacterial density of

6x107/ml, 6x106/ml, 6x105/ml, 6x104/ml, 6x103/ml, 6x102/ml, 60/ml, 6/ml prior to incubation were 9/20 replicate (45%), 9/20 (45%), 3/20 (15%), 1/20 (5%), 0/20 (0%), 0/20 (0%), 0/20 (0%), 0/20 (0%), respectively. After 24 hours of incubations were 20/20 replicate (100%), 18/20 (90%), 11/20 (55%), 8/20 (40%), 4/20 (20%), 2/20 (10%), 0/20 (0%), 0/20 (0%), respectively. From the DNA extract data, it could be determined that this PCR method required a DNA concentration of 84 ng/µL. Results showed a 24-hours incubation was needed in order to detect psaA by PCR and with the initial bacteria density of 6x106 organisms/ml in the inoculum. The weakness of study was DNA extraction process not optimal, shown by the variability of DNA concentration in the extracts which affected the ability of PCR to detect psaA gene prior to incubation., Streptococcus pneumoniae (pneumococcal) detection can be done by culture and PCR methods. The purpose of this study was to determine the limits of psaA gene PCR in detecting pneumococcal inoculum prior to incubation and after 24 hours of incubation of liquid media. This experimental study used Streptococcus pneumoniae ATCC (American Type Culture Collection) 49619 which was grown on sheep blood agar. Ten mililiter of bacterial suspensions with initial density of 6x107/ml, 6x106/ml, 6x105/ml, 6x104/ml, 6x103/ml, 6x102/ml, 60/ml, 6/ml were inoculated into liquid media, BD BACTEC™ Plus Aerobic/F Culture Vials. Each bacterial density was inoculated into these 20 liquid medias. From each inoculated BD BACTEC™ Plus Aerobic/F Culture Vial, prior to incubation and after 24 hours of incubation, Gram staining, subculturing on sheep blood agar, and psaA gene PCR were done. When pneumococcal colonies were found on sheep blood agar, the colonies were tested for catalase and optochin sensitivity. PsaA gene were determined as “positive” when amplicons with molecular weight 838 pairs of bases were found. Culture and PCR methods were determined as able to detect pneumococcus when > 60% of 20 replicates yield positive results. The psaA PCR positive result rate of initial bacterial density of 6x107/ml, 6x106/ml, 6x105/ml, 6x104/ml, 6x103/ml, 6x102/ml, 60/ml, 6/ml prior to incubation were 9/20 replicate (45%), 9/20 (45%), 3/20 (15%), 1/20 (5%), 0/20 (0%), 0/20 (0%), 0/20 (0%), 0/20 (0%), respectively. After 24 hours of incubations were 20/20 replicate (100%), 18/20 (90%), 11/20 (55%), 8/20 (40%), 4/20 (20%), 2/20 (10%), 0/20 (0%), 0/20 (0%), respectively. From the DNA extract data, it could be determined that this PCR method required a DNA concentration of ≥ 84 ng/µL. Results showed a 24-hours incubation was needed in order to detect psaA by PCR and with the initial bacteria density of 6x106 organisms/ml in the inoculum. The weakness of study was DNA extraction process not optimal, shown by the variability of DNA concentration in the extracts which affected the ability of PCR to detect psaA gene prior to incubation.]