Pengembangan Imunosensor Elektrokimia dengan Screen-Printed Carbon Electrode (SPCE) dan Bioreseptor IgG Anti-rLipL32 Antibodi untuk Deteksi Leptospirosis pada Hewan = Development of Electrochemical Immunosensor with Screen-Printed Carbon Electrode (SPCE) and Bioreceptor IgG Anti-rLipL32 Antibody for Detection of Leptospirosis in Animals

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Abstrak

Latar Belakang: Diagnosis leptospirosis dengan microscopic agglutination test (MAT) memerlukan kultur hidup dan bersifat serovar spesifik, sedangkan polymerase chain reaction (PCR) memerlukan bahan dan peralatan yang mahal. Pengembangan metode diagnosis yang cepat, sensitif, murah dan mudah diaplikasikan masih sangat diperlukan. Penelitian ini bertujuan untuk mengembangkan imunosensor ektrokimia dengan screen-printed carbon electrode (SPCE) dan bioreseptor IgG anti-rLipL32 antibodi untuk deteksi leptospirosis pada hewan.

Metode: SPCE dimodifikasi dengan reduksi graphene oxide (SPCE/rGO) dan drop casting graphene oxide (SPCE/GO). IgG anti-rLipL32 antibodi diimobilisasi pada SPCE/rGO dan SPCE/GO dan kemudian di blocking dengan BSA/skim milk. Karakterisasi dilakukan dengan scanning electron microscope (SEM) dan differential pulse voltammetry (DPV). Kinerja imunosensor ditentukan dengan uji stabilitas, penentuan batas deteksi (LOD) dan kuantifikasi (LOQ), uji selektivitas dan analisis sampel spike urin sapi. Respon elektrokimia diukur dengan DPV menggunakan Palmsens Sensit Smart potentiostat dan software PStouch.

Hasil: Hasil SEM menunjukkan perubahan morfologi permukaan elektroda lebih kasar dan adanya partikulat yang mengindikasikan keberhasilan imobilisasi antibodi. Karakterisasi DPV menunjukkan peningkatan arus puncak pada modifikasi SPCE/rGO dan SPCE/GO, serta penurunan arus puncak secara bertahap setelah penambahan NHS-EDC, antibodi, dan blocking BSA/skim milk. Respon arus meningkat seiring peningkatan konsentrasi bakteri Leptospira. Kurva kalibrasi menunjukkan linearitas tinggi dengan persamaan I = 0,8495 (Leptospira) + 0,5402 dan R^2 = 0,9856. Batas deteksi dan kuantifikasi diperoleh pada I = 1,00 (4 sel/ml) dan I = 3,35 (2 × 10^3 sel/ml). Imunosensor stabil sampai 1 bulan pada 4oC, dapat mendeteksi Leptospira patogen dan tidak mendeteksi E. coli dan Staphylococcus aureus.

Kesimpulan: Imunosensor elektrokimia yang dikembangkan memiliki selektivitas dan stabilitas tinggi serta mampu mendeteksi Leptospira patogen dalam sampel spike urine sapi sehingga berpotensi sebagai metode alternatif untuk deteksi leptospirosis pada hewan dengan cepat, murah, dan mudah diaplikasikan di lapangan.
.....Background: Leptospirosis diagnosis by microscopic agglutination test (MAT) requires live culture and is serovar specific, while polymerase chain reaction (PCR) requires expensive materials and equipment. Development of a rapid, sensitive, inexpensive, and easy diagnostic method is still needed. This study aims to develop an electrochemical immunosensor with a screen-printed carbon electrode (SPCE) and IgG anti-

rLipL32 antibody bioreceptor for leptospirosis detection in animals.

Method: SPCE was modified with reduced graphene oxide (SPCE/rGO) and drop-casting graphene oxide (SPCE/GO). IgG anti-rLipL32 was immobilised on SPCE/rGO and SPCE/GO and blocked with BSA/skim milk. Characterisation was done by scanning electron microscope (SEM) and differential pulse voltammetry (DPV). Immunosensor performance was determined by stability testing, limits of detection (LOD) and quantification (LOQ) determination, selectivity testing, and cattle urine spike sample analysis. Electrochemical responses were measured by DPV using Palmsens Sensit Smart potentiostat and PStouch software.

Result: SEM results showed electrode surface morphology changes, which appeared rougher, and particulate presence indicated successful antibody immobilisation. DPV characterisation showed the peak current increased in the SPCE/rGO and SPCE/GO modification. It also gradually decreased with NHS-EDC, antibody addition, and blocking BSA/skim milk.Current response increased with increasing Leptospira bacteria concentration. The calibration curve showed high linearity with the equation $I = 0.8495$ (Leptospira) + 0.5402 and $R^2 = 0.9856$. Limits of detection and quantification were obtained at $I = 1.00$ (4 cells/ml) and $I = 3.35$ (2×10^3 cells/ml). The immunosensor was stable for 1 month at 4oC, could detect pathogenic Leptospira and did not detect E. coli and Staphylococcus aureus.

Conclusion: The developed electrochemical immunosensor has high selectivity and stability and can detect pathogenic Leptospira in cattle urine spike samples, so it has potential for detecting leptospirosis in animals quickly, cheaply, and easily applied in the field.