Optimasi ekspresi dan purifikasi protein P24, pol dan GP41, rekombinan HIV-1 CRF01-AE pada sistem ekspresi e-coli untuk pengembangan uji diagnostik recombinant immunoblot assay = Optimization of expression and purification of P24, pol and GP41, HIV-1, CRF01 AE recombinant proteins in e coli expression system for recombinant immunoblot diagnostic assay development

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Abstrak

Latar belakang: Metode konvensional untuk mengkonfirmasi infeksi HIV ialah Western blot. Namun, western blot memiliki keterbatasan yaitu kontaminasi dengan antigen selular manusia dan masalah perbedaan genetik di antara subtipe HIV-1 yang menyebabkan hasil indeterminate dan ketidakakuratan diagnosis infeksi HIV-1 subtipe CRF01_AE yang dominan di Indonesia. Pemeriksaan western blot yang tersedia di Indonesia ialah untuk diagnosis infeksi HIV-1/2 dan tidak bersifat spesifik strain.
Metodologi: Pada penelitian ini digunakan protein p24 rekombinan sebagai antigen pada western blot. Dilakukan optimasi ekspresi protein p24 rekombinan HIV-1 CRF01_AE pada Escherichia coli BL21CP dan purifikasi serta western blot untuk mendapatkan informasi awal mengenai reaktivitasnya terhadap serum ODHA di Indonesia. Optimasi ekspresi dilakukan terhadap lama waktu induksi, konsentrasi IPTG, dan suhu induksi. Purifikasi dilakukan dengan metode immobilized metal-affinity chromatography (IMAC) dan sistem purifikasi Ni-NTA [Qiagen] pada kondisi native dengan optimasi pada konsentrasi imidazole dalam wash buffer.
Hasil: Konfirmasi protein rekombinan dengan western blot menunjukkan bahwa ekspresi dan purifikasi protein p24 rekombinan telah optimal dan reaktif terhadap serum pasien HIV-1 positif di Indonesia.
Kesimpulan: Protein p24 rekombinan dari penelitian ini dapat dikembangkan untuk uji diagnostik western blot berdasarkan subtipe CRF01_AE yang dominan di Indonesia.
.....Background: Conventional method for confirmation of HIV infection is western blot. However, western blot has limitation of contamination by human cellular antigen and genetic diversity matter among the HIV-1 subtypes that showed indeterminate result and inaccuracy for the diagnosis of HIV-1 subtype CRF01_AE infection predominantly in Indonesia. The western blot available in Indonesia is for diagnosis of HIV-1/2 which is not strain spesific. This research performed the p24 recombinant protein as the antigen in western blot.
Methods: We conducted the optimization in expression of p24 recombinant protein of HIV-1 subtype CRF01_AE in Escherichia coli BL21CP and purification and the confirmation by the western blot to obtain initial information about the reactivity of this recombinant protein with ODHA (people with HIV/AIDS) in Indonesia. Expression optimization administered in the induction time, IPTG consentration used, dan the induction temperature. The purification of the p24 recombinant protein carried with the immobilized metal-affinity chromatography (IMAC) method in Ni-NTA purification system [Qiagen] in native condition with optimization in the imidazole concentration used in the wash buffer.
Result: The confirmation of recombinant protein by western blot showed the expression and purification of p24 recombinant protein has been optimized well and reactive with the Indonesian HIV-1 positive serum

patient.
Conclusion: This result indicated the p24 recombinant protein can be applied for the diagnostic assay development based on predominant HIV-1 subtype CRF01_AE in Indonesia.