

# 山羊傳染性化膿性皮膚炎活毒減毒疫苗 及抗體檢測試劑研發

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## 摘要

103年度於本所GMP藥廠完成一批山羊傳染性化膿性皮膚炎次單位疫苗、三批活毒減毒疫苗以及一批羊痘P32和山羊傳染性化膿性皮膚炎F1L和B2L之雙價次單位疫苗試製，並完成試製疫苗之安全、效力和病毒迷入否定等自家品管檢定試驗。以蔗糖、乳糖及山梨糖醇做為冷凍乾燥保護劑之活毒減毒疫苗，經4℃保存13個月後每劑量之病毒力價均無顯著差異。此外，於台中市太平區及霧峰區各一牧場進行試製活毒減毒疫苗田間試驗，經以F1L ELISA檢測結果，免疫9天後即可測得抗體陽轉現象，有效抗體力價可以持續180天以上。試製之羊痘和CE雙價次單位疫苗，經以每劑 20  $\mu$ g 和 60  $\mu$ g 肌肉注射免疫羊隻後，分別經以自行研發之P32、B2L和F1L ELISA套組檢測結果均可測出相關特異性抗體並能耐過兩種強毒的攻毒。次單位疫苗佐劑比較試驗，將重組蛋白分別與ISA70適當混合乳化後免疫 Balb/c 小鼠結果，僅一次免疫後即可獲得抗體明顯揚升。懷孕約3月齡母羊，經免疫活毒減毒疫苗後未引發流死產或皮膚病變，顯示試製疫苗對羊隻具有高度安全性。除了F1L和B2L外，將可能具有免疫原性的ORF88、ORF94

和ORF104蛋白基因架構於原核表現質體上，片段大小分別為783bp、591bp和273bp。經定序確認序列無誤後，將架構好的質體轉型到大腸桿菌中，以IPTG加以誘導表現，經四和六小時誘導後分別萃取出可溶性和不可溶性蛋白，經SDS-PAGE蛋白膠體電泳後再經西方墨點法並以抗組織胺之單株抗體加以辨認結果，可見ORF88、ORF94和ORF104重組蛋白。

# **Development of Contagious Pustular Dermatitis attenuated live vaccine and its antibody detection kit**

## **Abstract**

A batch of contagious pustular dermatitis (contagious ecthyma; CE) trial subunit vaccine, 3 batches of attenuated live CE trial vaccine, and one batch of bivalent trial subunit vaccine consisting P32 of goat pox virus and F1L and B2L of CE virus recombinant proteins have been manufactured in the GMP factory of our institute. Safety, efficacy and virus contamination tests have been carried out for all these trial vaccines. After keeping in 4°C for storage tests for more than 13 month, the virus titers of each CE live vaccine with stabilizer like sucrose, lactose and sorbitol, respectively showed no significant difference. Furthermore, field trials of attenuated live CE vaccine have been conducted in two private goat farms in Taichung, and the ELISA test based on F1L protein for the vaccinated animals showed that they became sero-conversion on 9<sup>th</sup> day post vaccination, and their effective titers could persist for more than 180 days. The bivalent subunit vaccine of P32, F1L and B2L recombinant proteins can provide goats protection against both pox and CE virus challenge after intramuscular injection of 20 or 60 µg per dose, and positive ELISA titers of vaccinated goat serum samples can be detected with home-made P32, B2L and F1L ELISA Kits, respectively. For adjuvant selection test for subunit vaccines, recombinant proteins were mixed and homogenized with ISA70 properly, and were injected into mice, and obvious sero-conversion happened, even with a single shot. The pregnant goats about 3 months showed no abortion and skin lesions after receiving attenuated live CE vaccine, and it means this vaccine was safely for goats. In this study, other antigenic protein genes of ORF88, ORF94 and ORF104 have been cloned into *E. coli* expressing plasmids. After transformation into *E. coli*, inducing with IPTG for expression, extraction of soluble and insoluble proteins, expression of ORF88, ORF94 and ORF104 were demonstrated in western blot with the anti-His tag antibody.