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**Title:** New enzyme linked immunosorbent assay using recombinant antigens from *Saccharopolyspora rectivirgula* for farmer's lung disease serodiagnosis

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**Body:** *Saccharopolyspora rectivirgula* (SR) is the main etiologic agent of the Farmer's Lung Disease (FLD). Serodiagnosis using immunoprecipitation techniques with crude antigens has proved effective but lacks of standardization. We aim at producing specific recombinant antigens (rAg) to develop a new standardized enzyme-linked immunosorbent assay (ELISA). A database of putative proteins was first specifically created after partially sequencing of SR. Subsequently, proteins were analyzed by two-dimensional electrophoresis and revealed by Western-blot analysis with serum from 9 patients with FLD in comparison with the serum of 4 exposed controls. Among the 77 visible spots, 42 were analyzed by mass spectrometry and 28 proteins were identified. Sixteen FLD-specific proteins were produced as rAg in *Escherichia coli*. ELISA with 16 rAgs were performed using serums of 20 FLD patients from France and Switzerland and 27 controls. Results were analyzed by receiver operating characteristics curve. Five showed an area under the curve (AUC) above 0.75 and a sensitivity equal or greater than 75%. (named SR1FA, SR9, SR13, SR17 and SR25). The protein performing the best (SR9) reached 80% sensitivity and 85% specificity with an AUC of 0.90. ELISA using rAgs specific from SR were effective for serodiagnosis of FLD. The use of antigen panels including rAg specific from other micro-organisms involved in FLD (*Aspergillus*, *Lichtheimia*, *Wallemia*) may increase the diagnosis performance of the ELISA. A prospective study including FLD patients from other countries

(Finland, Canada), exposed to other strains of SR, should be done for a large scale validation.