

## EXPRESSION OF SCHISTOSOMA JAPONICUN FATTY ACID BINDING PROTEIN GENE IN SILKWORM CELLS AND LARVAE \*

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### ABSTRACT

**AIM:** To express the fatty acid binding protein (Sj14FABP) gene of *Schistosoma japonicum* in the silkworm cells and larvae. **METHODS:** A 600 bp DNA fragment containing Sj14FABP gene was cloned into baculovirus transfer vector of pBacPAK His1 to construct recombinant transfer vector Sj14-pBac PAK His1. Coinfection was accomplished with this vector and *Bombyx mori* nuclear polyhedrosis virus (BmNPV) DNA in BmN cells. The recombinant virus of Bm-Sj14 was screened using dot-blotting. The BmN cells and silkworm larvae were infected with Bm-Sj14 to express Sj14FABP gene. Western blotting and ELISA were used to identify the antigenicity of the recombinant protein. **RESULTS:** Sj14FABP gene was successfully expressed in the BmN cells and silkworm larvae infected with Bm-Sj14. The product was a 18 kDa fusion protein. The yield in BmN cells was about 100 µg/1 × 10<sup>6</sup> cells and 33 µg/ml cell supernatant. In silkworm larvae, the product yield was 4 mg/ml haemolymph as well as 4.6 mg/g silkworm tissue. The recombinant protein could be recognized by Western blotting and ELISA using the sera from mice immunized with SWAP. **CONCLUSION:** Sj14FABP gene has been successfully expressed in BmNPV system and the product has high antigenicity.

**Key words:** *Schistosoma japonicum*, fatty acid binding protein, gene expression, recombinant BmNPV, vaccine

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## 改良厚血膜疟原虫染色法

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厚血膜检查疟原虫具有原虫浓集、易查找、省时及检出率高等优点,但按常规染色方法由于红细胞被溶解,原虫常在溶血过程中皱缩与变形,在鉴别上造成一定困难,近年我们经反复摸索,改良了厚血膜染色法,结果疟原虫形态较完整,易鉴别,效果较好。

### 染色液的配制与染色方法

1 染色液的配制 用甲醇 500 ml (AR), 加入吐温-80 10 ml 及甘油 10 ml, 摇匀, 然后加瑞氏染料 1.0 g 及姬姆萨染料(均为上海试剂三厂产品) 1.0 g, 美蓝 0.05 g, 充分振荡混匀溶解, 备用; 用 40% 甲醛 0.8 ml 加 pH 6.8 PBS 液至 100 ml 为 0.8% 甲醛 pH 6.8 PBS 液。临用时将上述染液与 0.8% 甲醛 PBS 液按 1:4 配成混合染液。

2 染色方法 取血 2 滴~3 滴置于洁净的载玻片中央, 涂制直径为 1 cm~1.5 cm 均匀的血膜, 血膜不宜过厚, 自然干透后, 滴加 0.8% 甲醛 PBS 液 6 滴盖满血膜, 溶血 5 min 左右, 待血膜成灰白色后, 再用同样大小的滴管滴加混合染液

4 滴, 充分混合染色 3 min, 流水冲洗, 晾干, 镜检。

### 结果与讨论

被疟原虫感染的红细胞保留残影, 呈淡红色, 尤其是晚期滋养体及裂殖体感染的红细胞残影更为明显, 其它红细胞多被溶解。疟原虫染色清晰, 其形态基本不变, 原虫细胞核呈紫红色, 细胞浆呈淡蓝色, 疟色素呈棕色或褐色, 空泡不着色或着色即淡, 区分清楚, 容易鉴别, 此片亦可长期保存。

本染液中, 含有染胞浆较好的瑞氏染料和染胞核较好的姬姆萨氏染料, 且吐温-80 为高分子非离子型表面活性剂, 能增加染料的溶解度, 减少染料的沉渣, 促进染料成熟, 又能在染色过程中, 促使红细胞膜和血小板破裂。0.8% 甲醛 pH 6.8 PBS 液能使疟原虫形态基本保持不变, 沉渣少, 背景干净、清晰, 原虫着色鲜艳, 容易鉴别, 此法明显缩短染色时间, 且温度的高低对染色无明显的影响。

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