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ภาคผนวก

ขั้นตอนการตรวจหา ANCA ใน น้ำเหลือง

1. การเตรียม substrate

การเตรียมนิวโทรฟิล โดยการนำ fresh whole blood 1 unit ที่ใส่ heparin เพื่อป้องกันเลือดแข็งตัว มาปั่นแยก RBC และ น้ำเหลืองออก ได้ส่วน ที่เป็น buffy coat นำส่วนนี้มา smear บน slide ให้ได้ เป็นแผ่นฟิล์มบางขนาดหลอดผ่านศูนย์กลางประมาณ 1 cm. นำมา fix ด้วย absolute ethanol ประมาณ 5 นาที ที่ 4-8°C นำขึ้นมาทิ้งไว้ให้แห้ง เก็บไว้ที่อุณหภูมิ -70°C เพื่อนำมาใช้ภายหลัง เมื่อจะนำมาใช้ต้องทิ้งไว้ที่ อุณหภูมิห้องจนครบไอ้น้ำหมดไปก่อน

2. การเตรียมน้ำเหลืองที่นำมาทดสอบ

นำน้ำเหลืองของคนไข้มาเก็บรักษาไว้ที่ -70°C เมื่อนำมาตรวจหา ANCA ให้นำมาทิ้งไว้ที่อุณหภูมิห้องก่อนจึงจะนำมาตรวจ

3. การทดสอบ

3.1 นำ slide ที่ smear แล้วทิ้งไว้ที่อุณหภูมิห้อง คลายความเย็นลง

3.2 นำน้ำเหลืองของผู้ป่วย และน้ำเหลือง ของผู้ป่วยที่มาโรงพยาบาลด้วยโรคอื่น (control) ที่แช่แข็งทิ้งไว้ที่อุณหภูมิห้องให้น้ำแข็งละลายจนหมด

3.3 นำน้ำเหลืองมาทำให้เจือจางให้ได้ความเข้มข้น ที่ 1:2, 1:16, 1:64, 1:256 ด้วย phosphate buffer solution (PBS)

3.4 นำน้ำเหลืองที่เจือจางแล้วมาหยดลงบนแผ่น slide ที่เตรียมไว้ให้ทั่วบริเวณที่ smear ทิ้งประมาณ 30 นาทีใน moist chamber

3.5 นำ slide มาล้างด้วย PBS 3 ครั้งๆ ละ 5 นาที

3.6 จากนั้นนำ slide มาทำปฏิกิริยากับ น้ำยา

Antihuman-IgG ที่มี สารเรืองแสงจับอยู่ โดยหยดให้ท่วม ในบริเวณที่ smear ไขว้ ทิ้งไว้ 30 นาทีใน moist chamber

3.7 นำ slide มาล้างด้วย PBS 3 ครั้ง ๆ ละ 5 นาที

3.8 นำ slide มา mount ด้วย glycerine ปิด cover slid

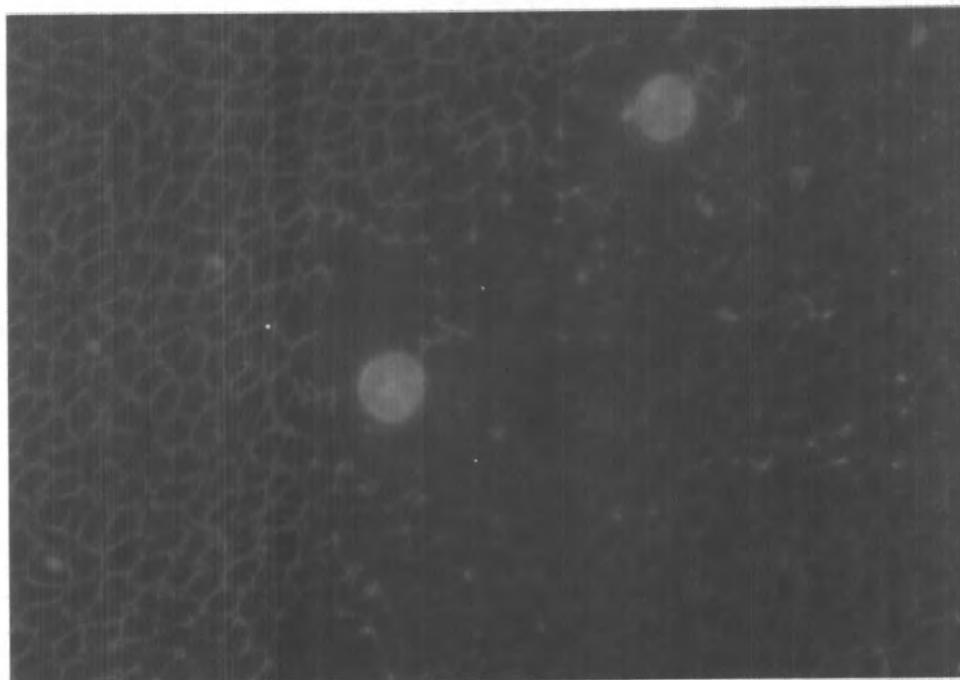
3.9 นำมาตรวจสอบด้วยกล้อง fluorescence microscope ที่นที หรือภายในไม่เกิน 48 ชม. แต่ต้องแช่ในตู้เย็นที่อุณหภูมิต่ำกว่า 8°C

3.10 บันทึกผล

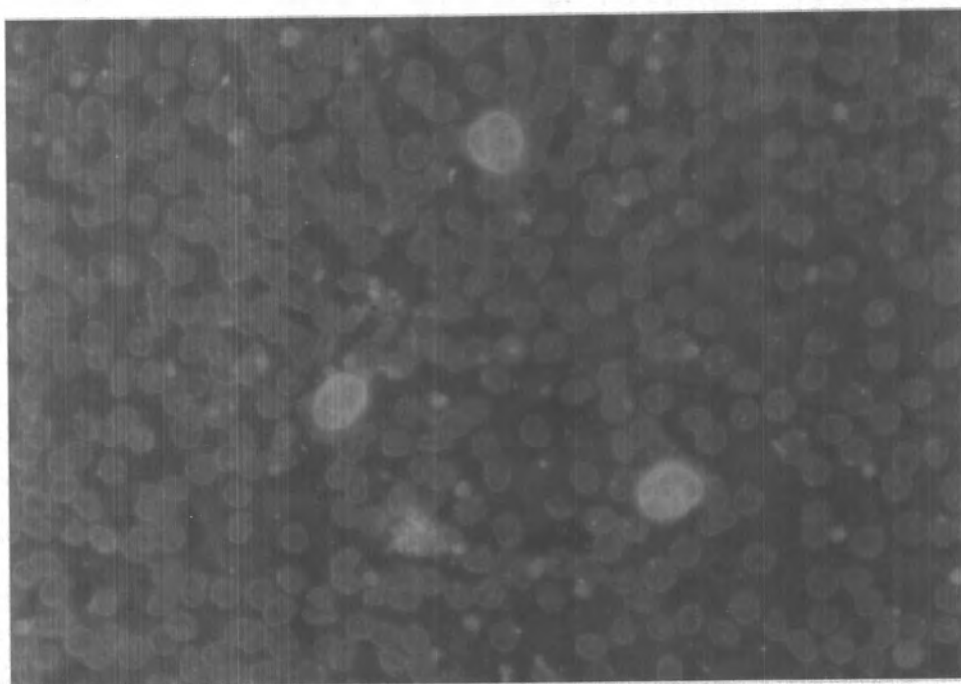


ประวัติผู้เขียน

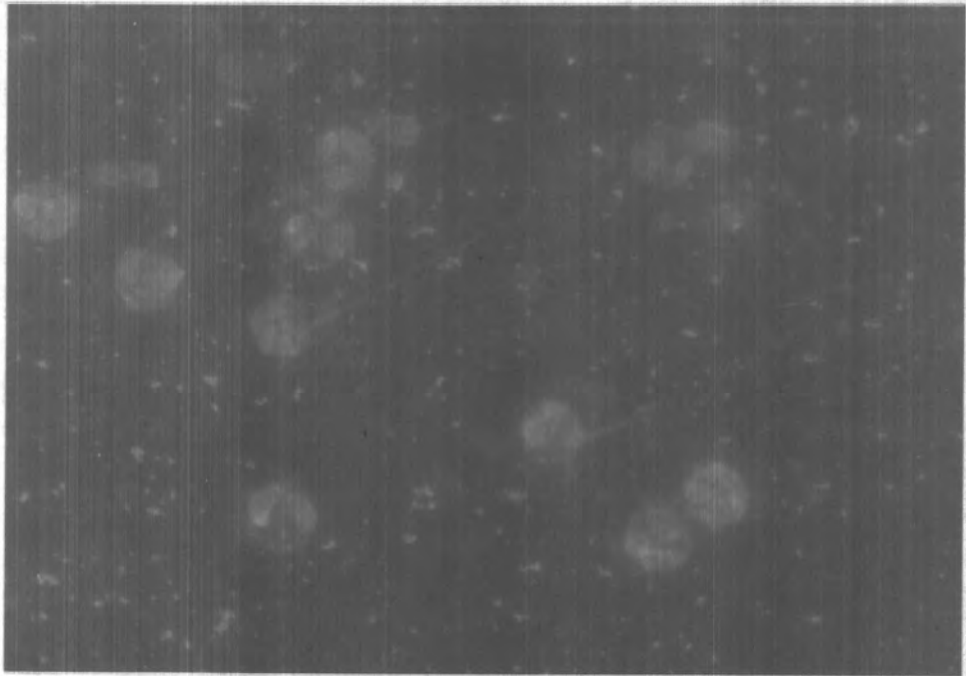
นายอิสสระ สอสุทไพบูลย์ เกิดเมื่อวันที่ 22 สิงหาคม พ.ศ. 2508
ที่กรุงเทพมหานคร จบการศึกษาระดับอุดมศึกษาจากโรงเรียนเตรียมอุดม
และสำเร็จการศึกษาปริญญาแพทยศาสตรบัณฑิต จุฬาลงกรณ์มหาวิทยาลัย
เมื่อปี พ.ศ. 2531



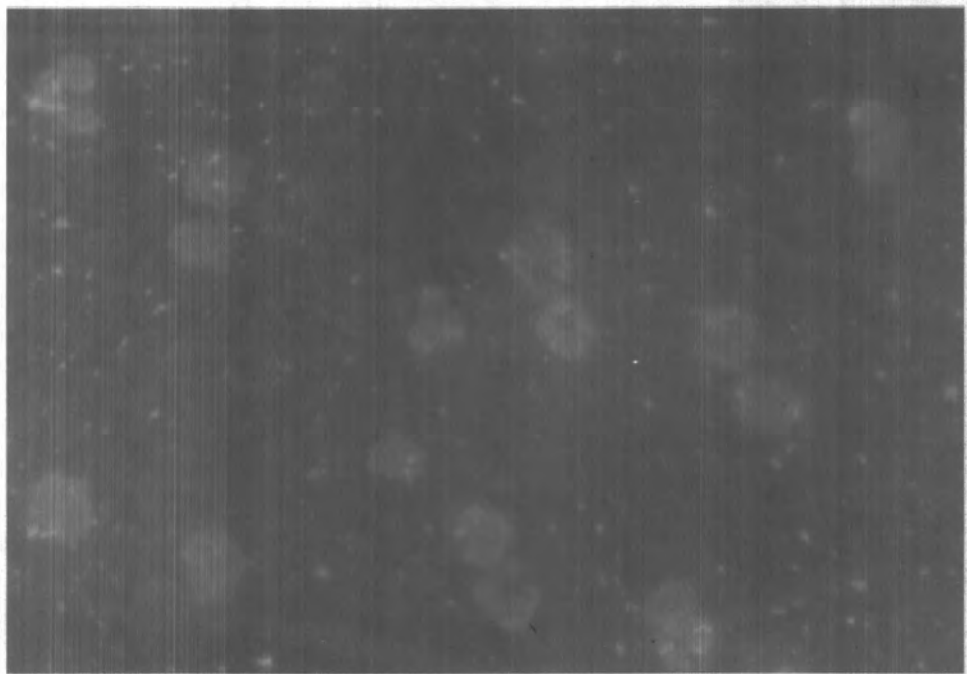
รูปที่ 2 แสดงลักษณะการเรืองแสงของ C-ANCA



รูปที่ 3 แสดงลักษณะการเรืองแสงของ C-ANCA



รูปที่ 4 แสดงลักษณะการเรืองแสงของ P-ANCA



รูปที่ 5 แสดงลักษณะการเรืองแสงของ P-ANCA