

## Evaluation of Chlamydia trachomatis IgA antibody in urethra of non-gonococcal urethritis patients.

Wimol Chanchaem\*

Pongpun Nunthapisud\*\* Ruthai Sakulramrung\*\*

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*An avidin-biotin immunoperoxidase assay has been set up for the detection of Chlamydial IgA antibody in urethral secretions as and adjunct to the diagnosis of Chlamydial infection in non-gonococcal urethritis (NGU) patients. The result of which were evaluated in comparison with the gold standard for diagnosis of current chlamydial infection, isolation of Chlamydia trachomatis. Of the 200 NGU patients studied C. trachomatis were identified in 69 (34.5%) upon isolation in McCoy cell culture. The avidin-biotin immunoperoxidase IgA presented a sensitivity, specificity, positive predictive value and negative predictive value of 79.7%, 77.8%, 65.4%, and 87.9% respectively. Thus an investigation of chlamydial IgA antibody in the secretion of NGU patients by avidin-biotin immunoperoxidase is applicable as an aid for the current infection with C. trachomatis.*

**Key words :** Chlamydia trachomatis, IgA antibody, non-gonococcal urethritis.

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\* Department of Biology, Faculty of Science, Ramkhamhaeng University.

\*\* Department of Microbiology, Faculty of Medicine, Chulalongkorn University.

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คณะผู้รายงานได้ทำการตรวจแอนติบอดีชนิด IgA ต่อเชื้อคลาไมเดีย ทราโคมาติส ในสิ่งคัดหลั่งจากผู้ป่วยด้วยโรคท่อน้ำสภาวะอักเสบ ซึ่งตรวจไม่พบเชื้อโกโนคอคคัส จำนวน 200 ราย โดยวิธี อิวติน-ไบโอติน อิมมูโนเปอร์ออกซิเดส และทำการประเมินผลเปรียบเทียบกับวิธีเพาะเชื้อ ซึ่งเป็นวิธีมาตรฐานจากการตรวจสอบด้วยวิธีเพาะเชื้อ พบผู้ป่วย 34.5% ที่ให้ผลบวก การตรวจการติดเชื้อโดยอาศัยแอนติบอดีชนิด IgA ต่อเชื้อคลาไมเดีย ทราโคมาติส ด้วยวิธี อิวติน ไบโอติน อิมมูโนเปอร์ออกซิเดส มีค่าความไว 79.7% และ ความจำเพาะ 77.8% เมื่อเทียบกับวิธีมาตรฐาน ดังนั้นวิธีการตรวจแอนติบอดีนี้จึงเป็นวิธีที่อาจนำมาใช้ช่วยประกอบการวินิจฉัยผู้ป่วยโรคท่อน้ำสภาวะอักเสบจากเชื้อชนิดนี้ได้

In recent years, Chlamydia trachomatis has been widely recognised as the most common sexually transmitted agent worldwide<sup>(1-4)</sup> It is associated with urethritis cervicitis, salpingitis, epididymitis, conjunctivitis and pneumonia.<sup>(2,5,6)</sup>

The definitive, most specific and reliable technique for diagnosis of chlamydial infection is isolation of organism in cell culture and it is the gold standard method of proving chlamydial infection.<sup>(1,2,4,7)</sup> However, it is time consuming in that it takes 3-5 days to get a result.<sup>(1,2,7)</sup>

Several studies have suggested that the presence of secretory IgA antibody was closely related to chlamydial infections and may be used as an aid to diagnosis.<sup>(8-11)</sup>

We evaluated the detection of urethral chlamydial IgA antibody in patients with non-gonococcal urethritis by the rapid immunoperoxidase test to investigate whether IgA antibody in secretions would be useful in diagnosis, the incidence of IgA antibody was determined and compared with isolation of C. trachomatis.

## Materials and methods

### Patients and specimens

The urethral specimens were collected from 200 males attending a Venereal Disease Clinic, Division of Infectious Disease Control, Department of Health. They were diagnosed as having non-gonococcal urethritis (NGU) by clinicians and confirmed by Gram stain of urethral specimen showing no gram negative diplococci.

The urethral secretions were collected prior to swabbing for culture, by inserting a small cotton swab in urethra. The secretion swab was placed into a vial containing phosphate buffer pH 7.4 to give a final dilution of 1 : 8.

A second swab was collected for isolation of C. trachomatis. The ENT swab (Medical Wire and Equipment, England) was inserted into urethra about 2-4 cm, then rotated. The swab was placed into Chlamydia transported media (2SPs). Then the specimens were transported in ice box (4°C) to the laboratory and kept in -70°C until tested.<sup>(12-14)</sup>

All 200 specimens were tested in the following manner :

- (i) Urethral swabs were tested in McCoy cells
- (ii) Urethral secretions were tested for chlamydial IgA antibody using an avidin-biotin immunoperoxidase assay (A-B IP)

### Isolation of C. trachomatis

An overnight growth of McCoy cells were prepared in 13 mm. coverslip flat bottom vials. Specimens were thawed rapidly; 4-5 sterile beads added to 2SP and vortexed for 60 seconds. Each tissue culture vial was inoculated with 0.5 ml. supernatant and centrifuged 3000 g, for 1 hour at 37°C. The supernatant was removed and replaced with 1 ml. of maintenance medium containing cycloheximide. After 48-72 hrs. incubation at 37°C the coverslips were fixed then stained with iodine and examined for presence of red-brown intracytoplasmic inclusion. A second and third passage was performed on all specimens containing no inclusion.

### Avidin-biotin immunoperoxidase technique

Preparation of chlamydial inclusion antigen slides

C. trachomatis serotype L2 provided by the Armed Forces Research Institute of Medical Sciences (AFRIMS) was inoculated on healthy confluent McCoy cell monolayer and incubated for 48 hrs. at 37°C then subpassaged until 30-80% infected cell culture was obtained. It was then trypsinized with 1% trypsin solution and suspended in growth medium to the concentration of  $2 \times 10^5$  cells /ml, thirty microliters of the suspension was used to coat on each well of 10 well teflon coated slides by further incubated for 24 hrs. in moist chamber.

### Avidin-biotin immunoperoxidase assay <sup>(15,16)</sup>

Frozen inclusion antigen slides were thawed, washed in PBS pH 7.4 and airdried before reacting with 10 ul. of patients secretion (1 : 8 dilution). The slides were incubated at room temperature for 45 minutes then washed in PBS and airdried. Ten microliters of goat anti-human IgA-biotin (Sigma, USA) at dilution 1 : 60 was applied, incubated at room temperature for 45 minutes then washed and airdried, followed by 10 ul. of peroxidase conjugate biotin-avidin complex (Sigma, USA) at dilution 1:80, incubated at room temperature for 45 minutes again. After washed and airdried, a final 10 ul. of substrate/chromogen solution was applied and incubated at 37°C for 30 minutes. The slides were then washed, airdried and mounted in 10% PBS pH 7.4 in glycerol. They were examined under a light microscope (10x,40x)

Positive results : A positive reaction was where the C. trachomatis inclusion was stained a dark blue colour and the McCoy cells were colourless.

Negative results : A negative reaction was indicated when all cells were colourless both in cytoplasm and inclusion.

### Statistical analysis

Two x Two contingency tables were utilized for the statistical analysis. The terms sensitivity, specificity, positive predictive value and negative predictive value of the tests have been used according to following definitions.

**Sensitivity :** the percentage of positive results of test in patients with positive gold standard test, isolation of C. trachomatis

**Specificity :** the percentage of negative results of test in patients with negative gold standard test, isolation of C. trachomatis

**Positive predictive value :** the probability that a patient who had a positive test result would be positive by gold standard test, isolation of C. trachomatis

**Negative predictive value :** the probability that a patient with a negative test result would be negative by gold standard test, isolation of C. trachomatis

### Results

#### Isolation of C. trachomatis

Of the 200 patients studied, 69 patients (34.5%) has Chlamydia isolated on culture.

#### Chlamydial antibody in urethral secretion

Of the 200 urethral secretions tested, 84 (42.0%) were positive for chlamydial IgA antibody by avidin-biotin immunoperoxidase.

The following table shows the results of A-B IP as against isolation of C. trachomatis from the urethra.

The sensitivity, specificity, positive predictive value and negative predictive value were 79.71% 77.86%, 65.48% and 87.93% respectively by avidin-biotin IP. Though sensitivity and specificity to detect C. trachomatis of this test are not such a high value to be used as single diagnostic tool but should be helpful to aid or support the diagnosis. The negative predictive value is also an interesting value. These should be in consideration for uses, especially where isolation of the organism not be done.

Comparison of urethral chlamydial IgA antibody demonstrated by A-B IP with isolation of C. trachomatis.

A-B IP	Isolation of <u>C. trachomatis</u>		total
	Positive	Negative	
urethral IgA Ab Positive	55	29	84
urethral IgA Ab Negative	14	102	116
total	69	131	200

Sensitivity 79.71%, Positive predictive value 65.48%  
Specificity 77.86%, Negative predictive value 87.93%

### Discussion

The presence of IgA antibody in the urethra is helpful in the diagnosis or exclusion of chlamydial infection. Though definite diagnosis of chlamydial infection is isolation of the organism in cell culture. This has been the gold standard method with a high specificity.<sup>(1,2,4,7,17)</sup> However, it is time consuming and is still not available in many laboratories. Isolation of C. trachomatis therefore been done mostly in large laboratories. This preliminary study demonstrates that urethral chlamydial IgA antibody by avidin-biotin immunoperoxidase can be used as an aid to diagnosis. The advantages of the test are its rapidness, simplicity and inexpensiveness which provide more facilitation to laboratories.

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