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Review Article

Diagnosis of Chlamydia infection in women

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Abstract

Objects: Chlamydia (*Chlamydia trachomatis*) is a common sexually transmitted infection that places a heavy burden on women and neonatal health. To avoid severe sequelae such as female infertility, ectopic pregnancy, neonatal infection, such as ophthalmitis, and chronic pelvic pain prompt and appropriate antibiotic treatment seems the best policy in treating this group of patients. However, adequate treatment is not easy because many factors can interfere with an early and rapid identification of Chlamydia infection, including complicated mixed microflora of the vagina and cervix, a nonuser-friendly detection system, and the time required for identification, even with the combination of specific complaints and a high level of clinical alertness. When dealing with a female patient in a point-of-care (POC) clinic, we need to find the best strategy to provide the most efficient way to detect this infection.

Materials and Methods: Totally five traditional methods and advanced technologies used for the diagnosis of Chlamydia infection in women were reviewed. A criterion proposed by World Health Organization with an acronym of ASSURED, representing affordable price, high sensitivity, high specificity, user-friendly design, rapid process, minimal equipment, and delivered-or-not, was used to reexamine these tools if they are the best tools. A multiplexed microchip-based immunoassay was evaluated as a potential tool. The ASSURED score was compared and a Chi-square test with a p value less than 0.05 was considered significant.

Results: Traditional methods, such as symptoms approach, microscopic examination, and microorganism culture that have been broadly used once, are affordable, simple, and equipment-free but their relatively low sensitivity and specificity limit their use as a test of POC setting for these infected women. On the other hand, advanced technologies, such as antigen detection by immunoassay and nucleic acid amplification tests, have contributed to major progress in the diagnosis of Chlamydia because of its accuracy, convenience, and time saving. However, nucleic acid amplification tests are too expensive, so they cannot be accepted as a screening tool in a developing country. The only significant finding with p value less than 0.01 was achieved when a more sensitive immunoassay system developed successfully as a test of POC setting.

Conclusions: Eventually, advances in laboratory techniques will satisfy our needs to detect Chlamydia infection economically and instantly. Microarray chips might be a relatively rapid, easy, inexpensive, and sensitive tool to detect many pathogens, including Chlamydia, using a one-time vaginal sampling process, which might make a POC policy possible.

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Keywords: Chlamydia; Rapid diagnosis; Sexually transmitted infections

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Introduction

Lower genital tract infection in women is the most common disease in gynecological clinics [1]. Most of these infections are caused by mixed pathogens. Treatment is frequently neglected or delayed [2], especially for sexually transmitted infections (STIs), resulting in subsequent fulminant infection (pelvic inflammatory diseases) [3]. STIs are also some of the most common urgent diseases in the emergency department. Although intensive treatment is used, some STIs are ultimately complicated with severe sequelae, resultant socioeconomic problems, infertility, ectopic pregnancy, preterm labor, and chronic pelvic pain [4]. Therefore, it is important to identify these highly invasive pathogens accurately and rapidly. Prompt and appropriate antibiotic treatment can lead to patients avoiding most of these complications.

To make an early diagnosis of STIs, familiarity with the diseases, a high degree of suspicion, and an easy-to-use diagnostic method are important. Because of the development of artificial reproductive techniques and the well-recognized adverse effects of Chlamydia infection on fertility, the polymerase chain reaction method has been used in the diagnosis of Chlamydia infection for decades. However, the diagnosis of other STIs is still often ignored because of the lack of a formal and organized training [5], the lower incidence in the general population, nonspecific symptoms or signs of infection, and the rare discomfort of the infected women. Of course, lack of simple, accurate, and easy-to-use diagnostic methods further attenuates our attention to these detrimental infections in women.

According to the estimate of the World Health Organization (WHO) [6], more than 340 million cases of curable STIs occur annually worldwide, and the sequelae of Chlamydia infection in women, such as the facilitation of HIV transmission, ocular infections of the newborn, disseminated infection and infertility, are severe and profound. Nearly blind use of antibiotics for *Chlamydia trachomatis* infection enabled resistant strains to spread widely and rapidly. All of these factors contribute to the necessity of revisiting Chlamydia infection.

In the general population, the estimated prevalence of Chlamydia infection is around 1.0–12% [7–9], and it increases up to 35% in specific high-risk groups [10], such as sex workers [10], those at low socioeconomic levels [6], or groups with factors, such as young age, black ethnicity [11], frequent sexual contacts [12], and cases with some specific symptoms, such as mucopurulent cervical discharge and lower abdominal pain [9,12]. Different incidence will determine the variable screening power of a diagnostic tool.

Several methods are used to establish a Chlamydia infection. These tools will be reexamined and compared according to the "ASSURED" criteria proposed by the WHO [13] and hopefully help the physicians revisit this common sexually transmitted disease and reconsider the necessity of a more suitable diagnostic tool to provide a point-of-care (POC) service [14]. ASSURED standards for a POC tool mean affordable price, high sensitivity, high specificity, user-friendly design, rapid process, minimal equipment, and delivered-or-not [15].

Material and methods

Totally five traditional methods and advanced technologies used for the diagnosis of Chlamydia infection in women were enrolled, including symptoms approach, microscopic examination, microorganism culture, immunoassay, and nucleic acid amplification tests (NAATs). The recently published articles (search terms, including Chlamydia, diagnosis, and technology) on the PubMed were reviewed. A criterion proposed by WHO with acronym of "ASSURED" was used to reexamine these tools. According to the characteristics of Taiwan's gynecologic practice, a scoring system quoted from Huppert et al [15] was modified and presented in Table 1. The total score divided by 15 was designed as ASSURED efficiency. The difference of this value among different tools was evaluated with Chi-square test (SPSS Version 12.0, SPSS Inc., Chicago, IL, USA), and a p value less than 0.05 was considered significant. A potential microchip-based detection system was supposed to increase the sensitivity and specificity of an immunoassay. Statistical assessment will include this particular tool.

This detecting system was inspired by the advances in high-throughput assays and microarray chip fabrication. It also included the liposomal nanovesicles technique to build up a signal amplifying system and increase the sensitivity. The details of this system are simplified and illustrated as Fig. 1. A liposome composed of outer surface bilayer and inner vesicle contains several hundred thousand fluorescent dyes, which amplify the fluorescent signals. On the other hand, antibody microarray is an excellent tool for multiplex detection of analytes (Fig. 2). Besides the benefits of high-throughput and multiplexed immunoassays, the amount of sample can be minimal.

The preliminary unpublished data from our laboratory show that the sensitivity limit of this system for Chlamydia antigen in the laboratory is around 6.0 μg (Fig. 3). The estimated cost for each chip is US$3, if 200 chips are fabricated at the same time.

Table 1
ASSURED scoring system quoted from Huppert et al [15] and modified according to the practice in a developing country

Score	Affordable (US$)	Sensitivity (%)	Specificity (%)	U	Rapid (h)	Robust	E-free	Delivered
0	>35	<65	<65	No	>3	No	No	No
1	<35	>65	>65	Yes	<3	Yes	Yes	Yes
2	<21	>80	>80		<0.5			
3	<7	>95	>95					

E-free = equipment-free; U = user-friendly.

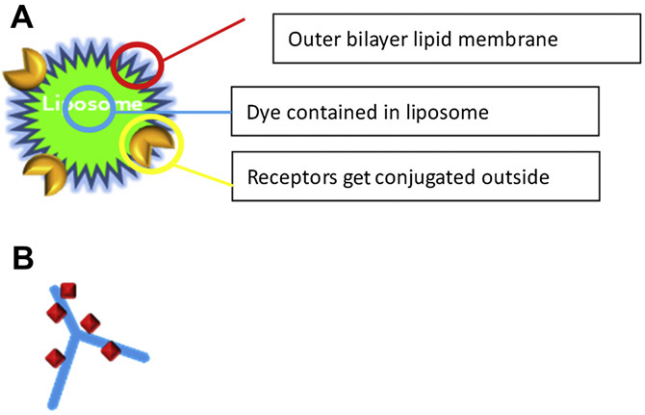

Fig. 1. The structure of the liposomal nanovesicles and detection antibody. (A) Liposomal nanovesicle: outer bilayer lipid membrane; (B) Detection antibody conjugated with biotin.

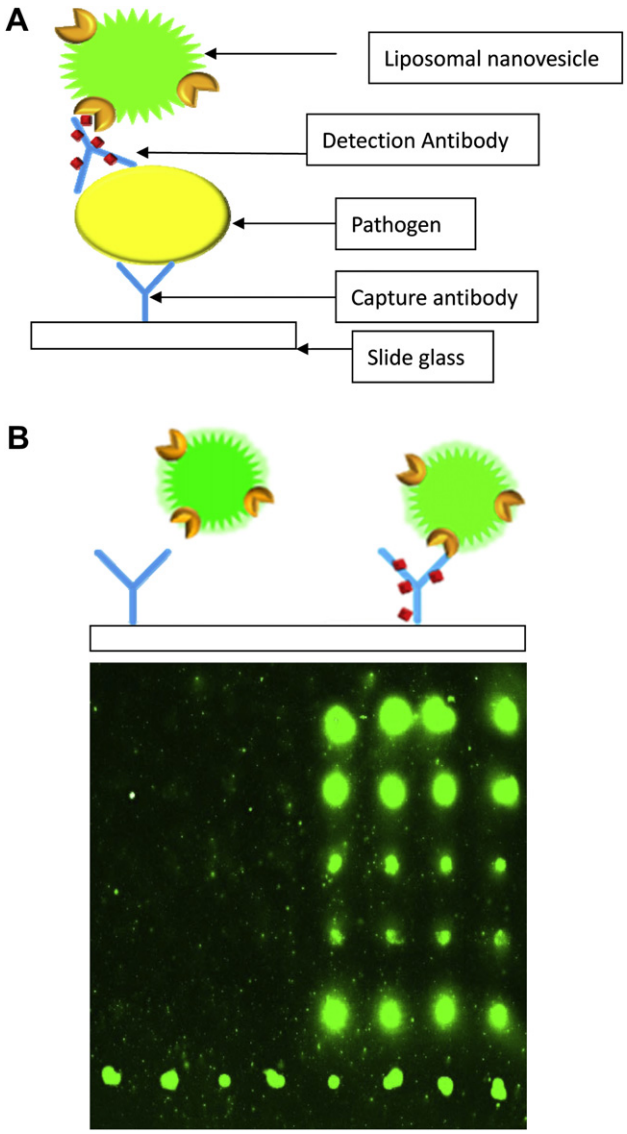

Fig. 2. The detecting procedure of microorganisms by microarray chip and examples. (A) The detection procedure. (B) Examples of the detecting system with original antibody and conjugated antibody on glasses showing positive detection of latter.

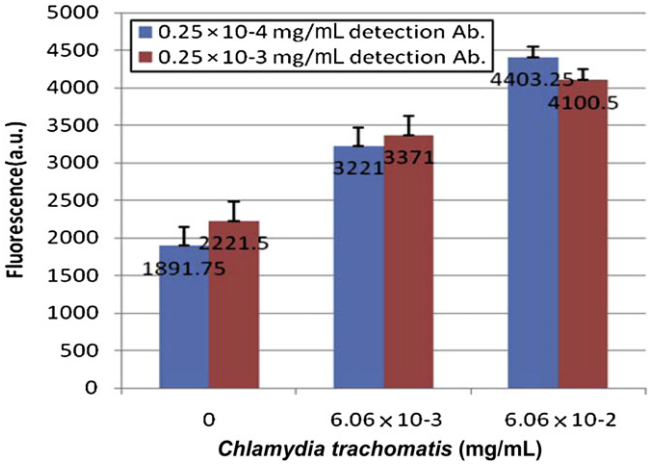

Fig. 3. The dose response table for Chlamydia antigen detection. Ab = antibody.

Results

The application of the revised ASSURED scoring system on the tools was summarized in Table 2. The affordable item was modified according to the situation of a developing country. In Taiwan, the cost of US$7 (NT 200) of an ordinary test was paid by government insurance (National Health Insurance), but for those noninsurance patients, US$3 (NT 1000) was requested. Sensitivity and specificity data from the references are presented in the Discussion section. User friendly is considered as the accessibility of training for an ordinary laboratory staff in Taiwan. Time consumed for the result was within 30 minutes like other ordinary tests in Taiwan or within 3 hours before the cease of an outpatient section. Robust score depends on the necessity to freeze and transport the samples. Unfortunately, NAATs for Chlamydia in Taiwan are almost not robust according to this definition. Equipment-free here meant the ease of sample collection. Finally, the delivered-or-not item is determined by whether it is available in ordinary clinic of Taiwan.

The statistics results of the advanced tools were summarized in Table 3. The only significant difference was identified between NAATs and the developing immunoassay system with a p value less than 0.01 when their sensitivity was more than 85% and specificity more than 95%.

Discussion

To clarify the value and limitation of the recently used tools for diagnosis of Chlamydia infection in female population, each tool has been extensively reviewed before the further discussion.

Method 1: syndromic approach

In a society with a lower socioeconomic level and limited laboratory resources, this may be the only possible method for the diagnosis of cervical infection. This subjective and non-consistent method depends on thorough surveillance of various symptoms caused by different and multiple pathogens in individuals with various physical-medical conditions. It is

Table 2
The results of application of scoring system (as Table 1) on different tools in Chlamydia detection

Tools	Affordable (US$)	Sensitivity	Specificity	U	Rapid	Robust	E-free	D	Total
Syndromic	3	12–83% (0)	43–73% (0)	1	2	1	1	1	9
Gram's stain	3	30–50% (0)	99–100% (3)	1	2	1	1	1	13
Culture	3	50–60% (0)	93% (2)	1	0	0	1	1	8
NAATs	0	91–100% (3)	98–100% (3)	0	0	0	1	1	8
Immunoassay	2	50–85% (1)	90–95% (2)	1	1	1	1	0	9
Microchip	2	90% (2)	95% (3)	1	1	1	1	1	12

D = delivered-or-not; E-free = equipment-free; NAATs = nucleic acid amplification tests; U = user-friendly.
The values inside the parenthesis are the calculated values ranging from 0 (the lowest value) to 3 (the highest value).

nearly impossible to identify the pathogen simply from the symptoms. Moreover, many victims are totally asymptomatic. This explains its low sensitivity and specificity.

It is believed that Chlamydia infection is confirmed most likely if a mucopurulent discharge is present during the vaginal speculum examination [6]. However, mucopurulent discharge (nonclear, yellowish discharge from the endocervix), friability (easy bleeding), or tenderness when the cervix is touched with a swab, and a positive swab test (yellow discoloration of the swab when inserted in the endocervix), may predict only 50% of Chlamydia infection [16].

In different populations, such as patients in a family practice, a prenatal clinic, or a setting especially for sex workers, different prevalence determines the fluctuated sensitivity, specificity, and positive predictive value of various detection tools [12]. The average sensitivity and specificity rates for an approach by syndrome are no more than 40% [6].

When using a syndromic approach combined with a scoring system using risk factors, including age, marital status, dyspareunia, and discolored vaginal discharge, higher sensitivity (60–80%) and specificity (around 60%) were sporadically reported in certain groups of patients [12]. Despite the low sensitivity and specificity, it is still an easy, rapid, and economic tool for the diagnosis. However, symptoms and signs of vaginal discharge did not predict cervical infection, and a syndromic approach failed to identify infected women [14]. Screening tests based on specific diagnostic techniques for *C trachomatis* are necessary because most infected women are asymptomatic [17].

Method 2: Gram's stain

The spreading of samples from the cervix on a glass slide and staining with dyes seems an inexpensive and convenient screening method for the diagnosis of infection and malignancy. However, it is not recommended for the diagnosis of cervical infection by the WHO [6] because of its low detection rate, even when well-trained technicians are available [6]. Some have tried to raise the detection ability of Gram's stain by using the criteria of finding 10 or more polymorphonuclear leukocytes per high-power field in the Gram's stain. However, as an aid to diagnosis, a result with sensitivity less than 50% is disappointing [18]. This might also indicate the minimal role of Pap smear in identifying cervical infection.

This method was used to study sexually transmitted urethritis. It was recommended for patients who are diagnosed presumptively as having urethritis because it may be the only objective evidence of urethritis [19]. And adding this method as an adjunct to the diagnostic tests for urethritis increased the etiologic diagnoses from 37.5% to 79% [20]. Obviously, this handy method has a potential but limited use in establishing the diagnosis of Chlamydia infection.

Table 3
The statistical results of ASSURED coefficients

Tools	Total score	Coefficient (%)	*p*	
			1	2
NAATs	8/15	53	0.60	0.0098
Immunoassay	9/15	60	0.053	0.60
Microchip	12/15	80	0.0098	0.053

ASSURED = affordable price, high sensitivity, high specificity, user-friendly design, rapid process, minimal equipment, and delivered-or-not; NAATs = nucleic acid amplification tests.

Method 3: culture

Cell culture of Chlamydia involves delicate processing procedures and well-trained personnel. Although it was used as a standard to be compared with the results of other methods, such as Gram's stain [21], immunoassay, or NAATs [22] at one time, there is growing recognition that the culture is not 100% sensitive and, therefore, is not an acceptable "gold standard" for assessing newer diagnostic technologies [23].

The sensitivity of this method may achieve a level of around 80–90% with specialized culture medium and culture conditions, skilled staff, and at least 72 hours of culture time [24]. A high level of suspicion in an alert clinician is essential for choosing the right culture medium and condition, and a significant improvement in the culture rate can also be achieved by urgent transport and processing of the specimens in the laboratory.

Compared with the advanced NAATs, the culture method might sometimes provide more accurate information when the NAAT method is targeting a changing gene. Moreover, this is still the only method that provides information about antimicrobial agent susceptibility. As resistant strains become commoner, high quality culture methods will be needed to ensure a representative sample for susceptibility tests.

Method 4: immunoassay

Different types of immunoassay kits are used to rapidly detect Chlamydia infections [15]. These methods include

optical immunoassay [25], Clearview Chlamydia [26], QuickVue [27], and the Chlamydia rapid test [28].

Despite their lower sensitivity of around 50–85%, immunoassays, as a rapid POC test, might outperform other standard tests in populations with high sexual activity, such as sexual workers and/or in those with low return rates in developing countries [29]. If treatment can be given at the initial visit, the possibility of the onward transmission of *C trachomatis* can be minimized [28]. Because it is an easy-to-use method with rapid diagnosis, low cost and no requested microscopy, it might provide a simple and reliable alternative to NAATs in the detection of Chlamydia infections [28], especially in a developing country [22].

Method 5: NAATs

So far, NAATs have provided high specificity, around 95–100%, and the best sensitivity—around 95%—of all diagnostic methods [22]. There are also consistent data among different NAATs, such as the strand displacement assay (SDA, BD ProbeTec *C trachomatis/N gonorrhoeae* Amplified DNA Assay, Becton-Dickinson, Sparks, MD, USA) [30], APTIMA Combo 2 and APTIMA GC assays, ligase chain reaction (Abbott Laboratories, Abbott Park, IL, USA) [31], and Cobas Amplicor (Roche) [22]. With their extremely high sensitivity and specificity, they are considered the "gold standard" for diagnosis and are good with different samples, such as cervical swabs from the intrusive speculum examination or urine from the noninvasive route.

However, there are still some shortcomings with these methods. They can cross-react with other Chlamydia species and can be affected by specimen transport conditions. False-negative reports, compared with culture data, resulting from some research indicate the variation in the target sequence. Therefore, confirmation with another NAAT method, which may double the cost, has been recommended.

Because of their high cost, NAATs are not recommended as the only tool for screening. The use of a molecular testing strategy may be cost-effective when it is supplemented with microscopy and culture to provide prompt treatment and further microbial susceptibility testing [32]. Actually, NAATs are not only expensive and complicated but also dependent on highly trained staff and delicate equipment. These factors all hinder the wide use of these in developing countries; therefore, they might not be suitable as a diagnostic method in a POC setting [28].

Method 6: multiplexed microchip-based immunoassay

Advances in the fields include high-throughput assays and microarray chip fabrication [33,34] inspired the development of this system. It is possible to perform multiple immunoassays simultaneously with one minimal vaginal sampling. In addition, the liposomal nanovesicles technique might build up a signal amplifying system and increase the sensitivity rate of immunoassays [35,36].

The liposomal nanovesicles are excellent and easy-to-use signal carriers in biosense analysis [37,38]. Besides the benefits of high throughput and multiplexed immunoassays, the amount of sample can be lowered to dozens of microliters [34,39,40]. These advantages make the method easier and more convenient.

STIs involve not only the female reproductive organs but also the anus and oral cavity; therefore, plenty of different microorganisms and pathogens are found, including different kinds of bacteria and viruses from the cervix. In this situation, it is reasonable and convenient to use a single approach with a multiplexed method to detect all the common microorganisms in one test, either by culture [41] or targeting on nucleic acid [42]. For an inflammatory disease caused by various and multiple pathogens, detecting all with one multiplexed method is the best policy. A thorough and detailed therapeutic plan can be useful in eradicating this infection [41,42].

Traditional methods, including syndromic approach, Gram's stain, and culture, are affordable, simple, and equipment-free; therefore, they are acceptable based on scoring system in this study. In fact, these traditional tools are widely used in the routine practice, although these tools are not recommended as the only screening tool in POC setting because of their relatively low sensitivity. We recommended that these traditional methods might be used as an adjunction role when combined with other tools.

In contrary, advanced technologies, such as antigen detection by immunoassay and NAATs, have contributed to major progress in the diagnosis of Chlamydia with extremely high sensitivity and specificity. NAATs are actually viewed as the new gold standard for detection of Chlamydia infection. Providing other advantages, such as time saving and easy sampling, NAATs are good candidates for a POC setting. However, the high cost and complicated equipments hinder their application in low socioeconomic districts or developing countries. On the other hand, although immunoassay is much cheaper and simpler than NAATs, its use as a rapid test is limited because of their relatively lower sensitivity. A more sensitive immunoassay system seems to overcome this limitation and fit the demands of a POC setting.

The best strategy for the diagnosis of Chlamydia infection should be based on an ideal POC tool with results available within 3 hours, followed by immediate treatment and contact tracing. This will also efficiently reduce the risk of persistent infection and onward transmission. To achieve our goal of an accurate diagnostic method for infectious diseases, such as Chlamydia or Gonococcus [43], advances in techniques to aid the detection of pathogens should be encouraged and supported [15].

NAATs supplemented with microscopy and culture are considered as a best choice in the ideal setting of a developed country [32]; because of above mentions, NAATs supplemented with microscopy and culture were inferior to the immunoassay methods, according to our POC scoring system. Particularly, in settings where laboratory facilities are not fully available, such as in a developing country or in high-risk populations where return rates are low, rapid tests using immunoassay may be a more effective way of diagnosing Chlamydia infection [28]. The optimal use in these settings requires the development of

rapid tests that are simpler and cheaper. An easy, fast, inexpensive, high-throughput, nanoscale-sensitive, and multiplexed detection system might be the answer. A clinician will be able to detect the different pathogens causing cervical and vaginal infections, including Chlamydia, immediately and accurately using a microarray-based immunoassay.

A POC setting, using a test that meets the criteria of ASSURED to provide an immediate diagnosis and to initiate prompt and appropriate treatment, seems to be our best policy in facing Chlamydia infection.

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