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Review Article

Are we satisfied with the tools for the diagnosis of gonococcal infection in females?

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Abstract

Gonorrhea (*Neisseria gonorrhoeae*) is a common sexually transmitted infection in women, with a heavy burden on female and neonatal health, because sequelae occur, such as female infertility, ectopic pregnancy, neonatal ophthalmitis and infection, and chronic pelvic pain. Prompt and appropriate antibiotic treatment can cure infection and avoid complications. However, adequate treatment is not easy, because early and rapid identification of gonorrhea is interfered with by many factors, including the complicated mixed microflora of the vagina and cervix, non-user-friendly culture systems, and lack of immediate availability of results, even with a combination of subjective complaint and high clinical suspicion. A PubMed search was conducted using the major headings of "gonorrhoea and diagnostic tool" and "*Neisseria* gonorrhoeae and diagnostic tool", before the end of 2010. Recently available methods for the diagnosis of gonorrhea infection in women were included, including traditional tools and advanced technology. Traditional tools such as microscopic examination and microbial culture have been used broadly; unfortunately, they have relatively lower specificity or sensitivity, and most importantly, "see-and-treat" is impossible for these infected women. Advances in technology, such as antigen detection by immunoassay and nucleic acid amplification tests (NAATs), have achieved major progress in the diagnosis of gonorrhea, because of their accuracy, convenience and time-saving aspects. However, NAATs are expensive, making their acceptance impossible in developing countries. Detection of pathogens including *N. gonorrheae* using microarray chips is viewed as a possible solution, because it is a relatively rapid, easy, inexpensive and sensitive tool, which makes an "identify-and-treat" or point-of-care policy possible. A rapid and affordable tool with high sensitivity and specificity for detection of gonorrhea in developing countries is still not available at the time of writing. To make a point-of-care policy possible, advanced technology for aiding diagnosis of gonorrhea is encouraged and appreciated.

Keywords: Microarray; *Neisseria gonorrhoeae*; point-of-care; rapid diagnosis; sexually transmitted infections

1. Introduction

Lower genital tract infection in women is the most common disease in gynecological clinics.[1] The majority of these infections are caused by mixed pathogens. Treatment is frequently neglected or delayed,[2] especially for sexually transmitted

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infections (STIs), resulting in subsequent fulminant infection (pelvic inflammatory diseases).[3] STIs are also some of the most common serious diseases in the emergency department. Even though 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, since prompt and appropriate antibiotic treatment can lead to patients avoiding the majority of these complications.

To make an early diagnosis of STIs, familiarity with the diseases, a high degree of suspicion and easy-to-use diagnostic methods are important. Chlamydia infection may be one of the most well-known examples, because of the development of artificial reproductive techniques and the well-recognized adverse effects of chlamydia infection on fertility. The polymerase chain reaction makes the diagnosis of chlamydia infection easier and quicker. By contrast, the diagnosis of *Neisseria gonorrhoeae* is often overlooked. The causes include the lack of a formal and organized curriculum in medical schools or house officer training,[5] the lower incidence in the general population, nonspecific symptoms or signs of infection, and rare discomfort of infected women. Of course, lack of simple, accurate and easy-to-use diagnostic methods further hinders our attention to this detrimental infection in women.

According to the estimate of the World Health Organization,[6] 62 million cases of gonorrhea occur annually worldwide, and the sequelae of gonorrhea infection in women, such as the facilitation of human immunodeficiency virus transmission, ocular infections of the newborn, disseminated infection and infertility, are severe and profound.[7] Nearly blind use of antibiotics for the *N. gonorrhoeae* infection has enabled resistant strains to spread widely and rapidly.[8] All of these factors contribute to the necessity of revisiting *N. gonorrhoeae* infection.

In the general population, the estimated prevalence of gonorrhea infection is around 0.7% to 7%,[9,10] and it increases to 15–35% in specific high-risk groups,[11,12] including sex workers or groups with different risk factors, such as young age, black ethnicity, frequent sexual contacts,[9] and cases with some specific symptoms, such as mucopurulent cervical discharge and lower abdominal pain.[10]

There are several tools that are used currently to establish diagnosis of gonorrhea infection.[2,7,8,10] This review attempts to describe and compare these methods, and hopefully help physicians better understand this common but overlooked sexually transmitted disease.

2. Syndromic approach

This is a subjective and non-consistent method for the diagnosis of a specific cervical infection. There should be various symptoms in a cervical infection caused by different and multiple pathogens in different persons with various physical conditions. It is nearly impossible to identify the pathogen simply from symptoms. Moreover, so many asymptomatic women are not aware of the risk of gonorrhea infection.

It is believed that gonorrhea infection is most likely confirmed if mucopurulent discharge is present during vaginal speculum examination.[6] Other factors include history of being a sex worker and tenderness induced by bimanual examination. However, mucopurulent discharge (non-clear, yellowish discharge from the endocervix), friability (easy bleeding) when the cervix is touched with a swab, or a positive swab test (yellow discoloration of the swab when inserted in the endocervix) predict only a 50% chance of gonorrhoeae infection.[13]

In different populations, such as patients from a family practice, in a prenatal clinic, or in a setting especially for sex workers, the sensitivity, specificity and positive predictive value of various methods differ due to fluctuating incidence.[10] The average sensitivity and specificity for an approach by syndrome are $<50\%$.[6]

Combined with a scoring system using risk factors,[14] such as age, marital status, dyspareunia, and discolored vaginal discharge, higher sensitivity (60–80%) and specificity ($\sim$60%) have been sporadically reported in certain groups of patients, using a syndromic approach.[10] Despite the low sensitivity and specificity, it is still an easy, rapid and economic tool for diagnosis. The syndromic approach remains the main diagnostic choice in most developing countries with limited laboratory facilities.[15]

3. Gram's stain

Spreading of smear samples from the cervix on a glass slide and staining with dyes seems an inexpensive and convenient screening method for diagnosis of infection and malignancy. Identification of intracellular Gram-negative diplococci under the microscope after Gram staining is considered an instant and standard finding, with specificity around 95%.[16,17] However, it is not recommended by the World Health Organization for diagnosis of cervical infection,[6] because of its low detection rate of near 30%,[18] 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 $\geq$10 polymorphonuclear neutrophils per high-power field. However, as an aid to diagnosis, a result with sensitivity $<50\%$ is disappointing.[16] This might also indicate the minimal role of Pap smear in identifying cervical infection.

4. Culture

Microbial culture is still accepted by some investigators as the gold standard for the diagnosis of gonorrhea infection,[19,20] and is used as a standard to be compared with the results of nucleic acid amplification tests (NAATs)[19] or other tools.[20] With a high specificity (95%) but a questionable detection rate (50%), this tool no longer satisfies the needs of modern medicine.

Optional conditions such as a CO_2-enriched (5–7%) and body-temperature (37 °C) environment, and an enriched medium such as modified Thayer–Martin medium or chocolatized medium will achieve a better culture rate.[21] These complicated conditions might explain the questionable sensitivity of this tool. To increase the detection rate, the high

suspicion of an alert clinician is essential for choosing the right culture medium and conditions, and a significant improvement in culture rate could also be achieved by urgent transportation to and processing of the specimens in the laboratory.[22]

Compared to the advanced NAATs, the culture method seems not sensitive enough to be the gold standard of diagnosis of gonorrheal infection. However, sometimes, the culture method might provide more accurate information when a NAAT is targeted on a changing gene.[23] Moreover, this is still the only method that provides information about antimicrobial agent susceptibility. Although resistant strains become increasingly prevalent, high-quality culture methods will need to be maintained to ensure a representative sample for susceptibility testing.[24] Moreover, the culture method can be used as a rescue approach when symptoms persist even after optimal treatment.

5. Immunoassay

Different immunoassay kits are used as a rapid method to confirm the genus in the *Neisseria* family. The products available on the market include GonoGen, GonoGen II, the Syva Micro Trak Direct Fluorescent-Antibody test, the Phadebact Monoclonal GC OMNI test, and the Gonobio-Test.[25] These tools are useful in telling us if the culture is *N. gonorrhoeae* or not. Some use this tool to detect possible infection from original cervical swabs directly. The sensitivity rates of currently available rapid tests are between 50% and 70%. The change of target antigen after routine use may decrease sensitivity after extinguishing the related subtypes, but improve the specificity up to 90–100%.[11,17]

In spite of their lower sensitivity, immunoassays, as rapid point-of-care tests, 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.[26] If treatment can be started at the initial visit, the possibility of onward transmission of *N. gonorrhoeae* can be minimized[7,18] Immunoassays are an easy-to-use method with rapid diagnosis, low cost and no requested microscopy, therefore, they seem more acceptable than other tools in developing countries.[11,17,18,20]

6. NAATs

To date, NAATs have provided high specificity of 95–100%, and the best sensitivity of all diagnostic methods, at around 95%.[19,20,22] There are also consistent data among different NAATs, such as the strand displacement assay (BD ProbeTec C trachomatis/N gonorrhoeae Amplified DNA Assay) APTIMA Combo 2 and APTIMA GC assays,[19] Ligase Chain Reaction (Abbott Laboratories, Abbott Park, IL, USA),[20] and Cobas Amplicor (Roche).[20] With their extremely high sensitivity and specificity, they are considered the gold standard for diagnosis[27] and good for different samples, such as cervical swabs from intrusive speculum examination or urine from the noninvasive route.[28]

However, there are still some pitfalls in using these tools. They can cross-react with other *Neisseria* species and can be affected by specimen transport conditions. False-negative reports, compared with culture data, indicate the variation in the target sequence. Therefore, confirmation with another NAAT method, which may double the cost, has been recommended.[23] In addition, the widespread use of NAATs might result in decreased isolation of *N. gonorrhoeae* and ignorance of possible resistant organisms.

Due to 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.[29] In fact, NAATs are not only expensive and complicated, but also dependent on highly trained staff and delicate equipment. These factors all hinder the widespread use of these techniques in developing countries.[11] Therefore, they might not be suitable as a diagnostic method in a point-of-care setting.[11,18]

7. Prospecting method

Advances in high throughput assays such as protein microarray chip fabrication[30] have made it possible to perform multiple immunoassays with one minimal vaginal sample. Up to several thousand proteins can be printed and evaluated on one slide simultaneously.[31] In addition, liposomal nanovesicles might be used to build up a signal amplifying system and increase the sensitivity rate of immunoassays.[32,33] This strategy has been used successfully for the detection of an infectious *Escherichia coli* strain, with a detection limit 100 CFU/mL,[32] and similar immunoassay techniques have been applied to detect several food-borne pathogens simultaneously, including *E. coli* O157:H7, *Salmonella enterica* serovar typhimurium, and *Listeria monocytogenes*, with detection limits between 100 and 15,000 CFU/mL.[33]

The liposomal nanovesicles are composed of outer-surface bilayer and inner vesicles; there are several hundred thousand fluorescent dyes contained within the vesicle (Fig. 1), which amplify the fluorescent signals. These are excellent and easy-to-use signal carriers in biosense analysis.[34] In addition, antibody microarray is an excellent tool for multiplex detection of analytes (Fig. 2). The illustrated procedures comprise several steps. Capture antibodies are first printed on the slide. After interaction with pathogens, detection antibodies

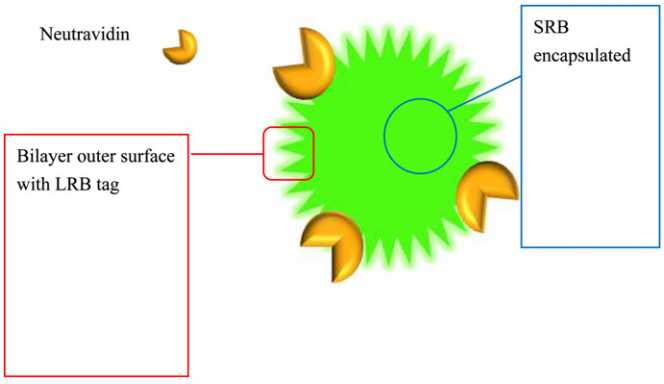

Fig. 1. The structure of the liposomal nanovesicles. SRB = sulfo-rhodamine B; LRB = lissamine-rhodamine B.

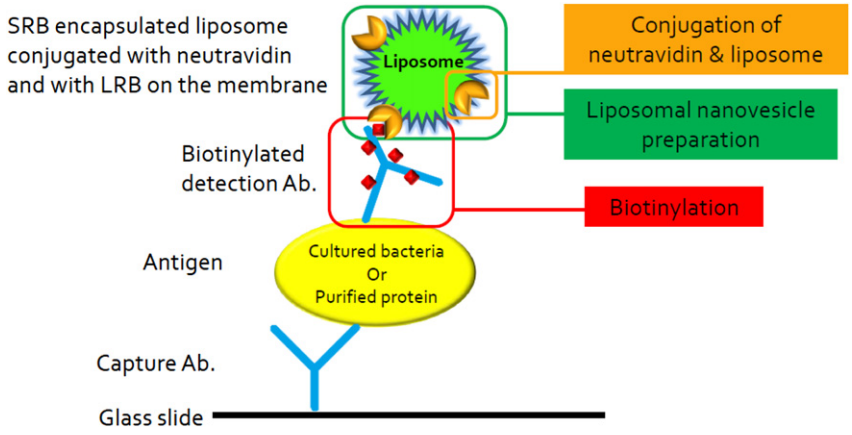

Fig. 2. The basic principle of detecting microorganisms by microarray chip. SRB = sulfo-rhodamine B; LRB = lissamine-rhodamine B; Ab = antibody.

conjugated with biotin are added. Finally, the amplifying liposome system is used to label the biotin.

Another advantage is the multiplexed nature of a microarray assay. STIs involve not only the conventionally named female reproductive organs, but also the anus or oral cavity; therefore, there are plenty of microorganisms and pathogens 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 nature to detect all the common microorganisms in one test either by culture[35] or targeting of nucleic acid.[36] For an inflammatory disease caused by various and multiple pathogens, detection of them all in one multiplexed tool is the best policy. Moreover, a thorough and detailed therapeutic plan can be useful in eradicating the infection.[35,36]

Besides the benefits of high throughput and being multiplexed, the amount of sample in this immunoassay can be lowered to tens of microliters.[30] These advantages make the method more convenient and easier to sue. The best part is that the estimated cost for each chip is US$3, if 200 chips are fabricated at the same time. Now, this technique is also being tested in our laboratory for the detection of gonorrhea infection. We hope to report the results in the near future.

In conclusion, a brief summary of available methods for the diagnosis of *N. gonorrhoeae* infection is listed in Table 1. Although the traditional methods have higher scores, the sensitivity of these tools is too low and is not acceptable for a screening method. In contrast, the NAATs show the best sensitivity and specificity; however, the application of NAATs to the clinical practice might not be cost-effective since the score, as shown in Table 1 is not high. This main defect limits their popularity, and they are not widely used in point-of-care settings. When considering a screening tool in a developing country, the tools reviewed above do not fit the routine clinical practice, which means that we are not still satisfied with these methods. To achieve the goals of accurate diagnosis and effective treatment of infectious diseases, such as gonococcal infection, advances in techniques to aid the detection of pathogens should be encouraged and supported.[24]

Factors determining the choice of screening tests for gonorrhea infection in women might include test sensitivity, specificity, available antibiotic susceptibility tests, ease of specimen collection, time, cost, degree of technique difficulty, and laboratory facilities.[21] NAATs supplemented with microscopy and culture remain the best choice in an ideal setting of a developed country.[24,29] However, in settings where laboratory facilities are not fully available, especially in a developing country or in high-risk populations where return rates are low, rapid tests using immunoassay may be the most effective way of diagnosing gonorrhea infection.[11,18] The optimal use in these settings requires the development of rapid tests that are simpler and cheaper.[7] An easy, fast, inexpensive, high-throughput, and nano-scale-sensitive multiplexed detection system might be the answer. A clinician might be able to detect the different pathogens causing cervical and vaginal infections, including gonorrhoea or chlamydiasis,[37] immediately and accurately using a microarray-based immunoassay. With a sensitive test for the point-of-care setting, a correct prescription of antibiotics will be made rapidly and confidently and over- or under-treatment can be avoided.

Table 1
Comparison and ranking scores of diagnostic tests for gonorrhea infection in women

Tools	Sensitivity[a]	Specificity[b]	Cost[c]	Time[d]	TRA[e]	FAC[f]	SPE[g]	SUS[h]	POC[i]
Syndromic	12–83 (1)	43–73 (1)	5	5	5	5	0	0	22
Gram's stain	30–50 (1)	99–100 (5)	4	4	4	4	0	0	22
Culture	56 (2)	95 (5)	3	1	3	3	0	1	20
Immunoassay	60–85 (2)	90–97 (4)	2	4	2	2	0	0	16
NAATs	91–100 (5)	98–100 (5)	1	2	1	1	1	0	16

[a]highest score, highest sensitivity, [b]highest score, highest specificity, [c]highest score, cheapest, [d]lowest score, shortest time, [e]lowest score, best training, [f]lowest score, best facilities, [g,h]bonus items with an extra score of 1, [i]total scores for the other items, higher ones might be better for developing countries, TRA = training of staff; FAC = laboratory facilities; SPE = spectrum examination; SUS = susceptibility test; POC = point of care setting.

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