Molecular diagnostic testing for infectious diseases using TMA technology

Craig S Hill

Molecular diagnostic tests based on nucleic acid amplification technologies (NAATs) have become widely established in clinical microbiology laboratories in recent years. The acceptance of these tests has been driven by the development of more accurate and less labor-intensive commercial assay kits by diagnostic manufacturers. Infectious disease diagnostic assays using transcription-mediated amplification (TMA) NAAT have become increasingly popular in many clinical microbiology laboratories. Recent technology developments have improved the performance and simplified the use of the TMA assays. These new technologies have been applied to the development of multiplex TMA tests to improve the testing accuracy for organisms, such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in clinical microbiology laboratories. TMA tests for HIV-1 and HCV have also led to improvements in blood bank testing which can improve the safety of the public blood supply.

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Gen-Probe, Inc.,
10210 Genetic Center Dr.,
San Diego, CA 92121 USA
Tel.: +1 858 410 8975
Fax: +1 858 410 8141
Craigh@gen-probe.com

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Nonamplified DNA probe tests have been used for many years in research laboratories. However, the relatively low sensitivity and high complexity of these 'home-brew' assays were a barrier to the use of the tests in the routine clinical laboratory for many years. Gen-Probe, Inc. (San Diego, CA, USA) developed the first FDA-approved, highly sensitive DNA probe tests suitable for use in the clinical microbiology laboratory. Assay sensitivity needed for clinical laboratory diagnostics was achieved by targeting ribosomal RNA (rRNA) rather than DNA and by the development of simple solution-phase hybridization methods. rRNA exists in thousands of copies in most microorganisms while target sequences in genomic DNA are present in a limited number of copies per organism [1]. The increased number of rRNA copies greatly increases the sensitivity of the assays because there are many more target molecules available for detection. Assay sensitivity and simplicity were further increased by the development and use of the hybridization protection assay (HPA) method for detection [2,3]. This is a homogeneous, chemiluminescent method utilizing acridinium ester-labeled DNA probes. The entire procedure occurs in a single test tube and no wash steps are required. The combination of rRNA targeting and HPA detection technology led to the first widely used DNA probe tests in the clinical laboratory for both culture confirmation as well as direct detection assays (TABLE 1). The assays are simple to perform and produce accurate clinical results in a few hours instead of the several days sometimes needed for biochemical identification. Analytical sensitivity is improved, allowing DNA probe assays to be used with samples containing low numbers of organisms. The detection limit of the HPA direct detection and culture confirmation assays has been estimated to be approximately 500–1000 microorganisms per assay.

Among the first commercial applications of the Gen-Probe nucleic acid probe technology were the AccuProbe culture confirmation assays. Several of the AccuProbe assays became the gold standard for the culture identification of microorganisms, such as *Mycobacterium tuberculosis* and *Mycobacterium avium* complex [4–6]. One of the first assays developed using HPA technology for the direct detection of organisms in clinical samples was the PACE 2 test. The PACE 2 assay for *Chlamydia trachomatis* (CT) and *Neisseria*

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gonorrhoeae (GC) was the first test ever produced to allow testing for both organisms from the same urogenital sample [7–10]. PACE 2 is currently the most widely used nonculture assay for detection of these organisms in the USA. The CT and GC assays also have been combined into a single assay that can simultaneously detect both organisms in a single reaction (PACE 2C) [11,12]. Another widely used direct assay is the Group A Streptococcus Direct assay for detection of *Streptococcus pyogenes* from throat swabs [13].

Some clinical samples contain too few microorganisms to be adequately detected by nonamplified DNA probe assays. Examples of these types of samples include respiratory samples from tuberculosis patients and urine samples from patients infected with CT. Although many different techniques can detect CT in cervical and urethral swab samples, these same techniques are not always able to detect this microorganism in urine with the required sensitivity. Limitations, such as these led to the development of clinical diagnostic assays utilizing nucleic acid amplification technologies (NAATs), for example polymerase chain reaction (PCR) [14]. Gen-Probe has developed an isothermal, transcription-based NAAT called transcription-mediated amplification (TMA) for application to clinical diagnostic needs. First-generation TMA assays for CT and *M. tuberculosis* have been cleared or approved by the FDA for use in clinical microbiology laboratories. The TMA technology has recently been enhanced with the development of APTIMA technology. APTIMA is a combination of three technologies consisting of TMA together with target capture specimen processing and the dual kinetic assay (DKA) detection technology.

Gen-Probe technologies

TMA

TMA is a target amplified, transcription-based amplification method developed to integrate with the Gen-Probe rRNA targeting and HPA technologies. The amplification process is isothermal and autocatalytic. Since the reaction occurs isothermally, a heat block or water bath is used instead of a thermal cycler. The kinetics of TMA are very rapid and billions of RNA amplicons are produced from a single target molecule in less than 1 h. TMA can be used with any type of nucleic acid target including rRNA, mRNA or DNA. The entire reaction occurs in a single test tube that greatly simplifies the processing. The TMA system is composed of three steps: sample preparation, amplification and detection.

Sample preparation

Disruption of the target microorganism is necessary to release the target nucleic acid into the assay mixture. Depending on the microorganism to be identified, this is performed either by chemical or enzymatic methods, or by mechanical means, such as sonication. The released nucleic acid is stabilized and the tubes are heated briefly at 95°C to denature the target nucleic acid and to inactivate infectious agents. The lysate now contains free nucleic acid that serves as a template for *in vitro* replication.

Table 1. Examples of commercially available DNA probe and TMA assays produced by Gen-Probe.

Direct probe hybridization protection assays
Bacteria
Group A streptococcus
Chlamydia trachomatis
Neisseria gonorrhoeae
Culture confirmation hybridization protection assay
Bacteria
Campylobacter
Enterococcus
Group B streptococcus
Haemophilus influenzae
Neisseria gonorrhoeae
Mycobacterium avium
Mycobacterium intracellulare
Mycobacterium avium complex
Mycobacterium gordonae
Mycobacterium kansasii
Mycobacterium tuberculosis
Streptococcus pneumoniae
Group A streptococcus
Staphylococcus aureus
Listeria monocytogenes
Fungi
Cryptococcus neoformans
Coccidioides immitis
Blastomyces dermatitidis
Histoplasma capsulatum
Transcription-mediated amplification assays
Mycobacterium tuberculosis
Chlamydia trachomatis
Neisseria gonorrhoeae
Chronic myelogenous leukemia (CML)[§]
HCV[§§]
HIV-1/HCV-1[§§§]

The direct probe and transcription-mediated amplification assays are used directly with processed samples. The culture confirmation assays are used with processed culture isolates. All assays are FDA cleared or approved unless otherwise noted. [§]Available as an ASR; [§§]Available as an ASR from Bayer, Inc.; [§§§]Blood bank test available from Chiron Corp.

Amplification

FIGURE 1 shows schematically an example of the TMA process, which uses two primers and two enzymes, RNA polymerase and reverse transcriptase. One of the primers contains a promoter sequence for RNA polymerase. In the first step of amplification, the promoter-primer hybridizes to the target rRNA at a defined site. Reverse transcriptase creates a DNA copy of the target rRNA by extension from the 3′ end of the promoter-primer. The RNA in the resulting RNA–DNA duplex is degraded by the RNAse H activities of the reverse transcriptase. A second primer then binds to the DNA copy. A new strand of DNA is synthesized from the end of the primer by reverse transcriptase, creating a double-stranded DNA molecule. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesized RNA strands can re-enter the TMA process and serve as a template for a new round of replication, leading to an exponential expansion of the RNA amplicon. Since each of the DNA templates can make 100–1000 copies of RNA amplicon, this expansion can result in the production of 10 billion amplicons in less than 1 h. The entire process is autocatalytic and is performed at a single temperature.

Detection

Detection of amplicon produced by the TMA reaction is performed by the HPA separation/detection process [2,3]. The process is begun by the addition of acridinium ester-labeled DNA probes that specifically hybridize to the target amplicon (FIGURE 2). A chemical reaction is used to selectively destroy the acridinium-ester label on the unhybridized probes. The label on the hybridized probes is protected from destruction within the double helix of the molecule. The final step of the assay is to put the reaction tubes into a luminometer, which automatically injects hydrogen peroxide and base into the tubes to produce the chemiluminescent signal. The signal is measured by the luminometer and expressed as relative light units (RLU). This is a homogenous assay format that requires no wash steps that could potentially spread contamination throughout the laboratory.

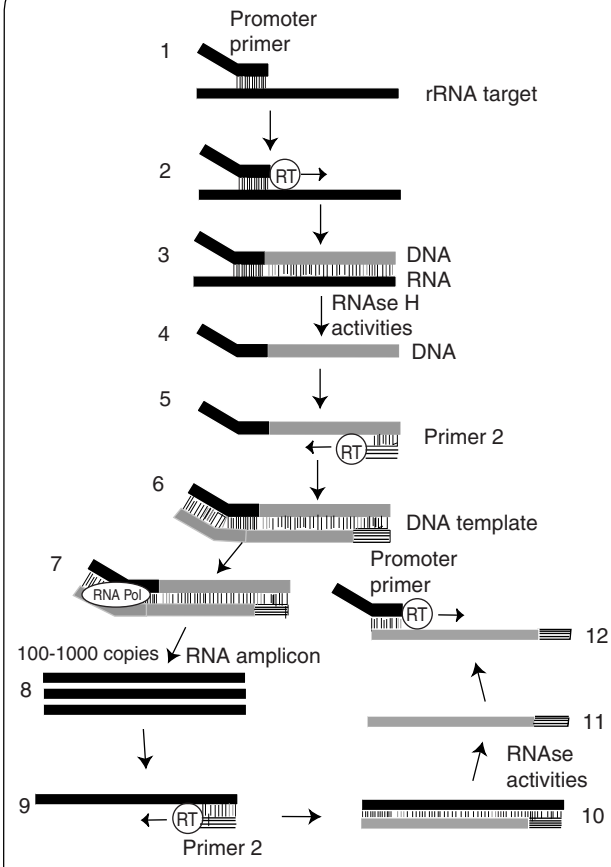

Figure 1. Diagram of the transcription-mediated amplification reaction (TMA). TMA uses two primers in the reaction. One of the primers contains a promoter sequence for RNA polymerase. In the first step of amplification, the promoter-primer hybridizes to the target rRNA at a defined site. Reverse transcriptase creates a DNA copy of the target rRNA by extension from the end of the promoter-primer. The RNA in the resulting RNA–DNA duplex is degraded by the RNAse H activities of the reverse transcriptase. A second primer then binds to the DNA copy. A new strand of DNA is synthesized from the end of the primer by reverse transcriptase creating a double-stranded DNA molecule. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesized RNA amplicons re-enters the TMA process and serves as a template for a new round of replication leading to an exponential expansion of the RNA amplicon. Since each of the DNA templates can make 100–1000 copies of RNA amplicon, this expansion can result in the production of 10 billion amplicon in 15–30 min.

APTIMA technology

The APTIMA technology is a second-generation amplification system consisting of target capture specimen processing, TMA and the HPA and DKA detection technologies. APTIMA technology was developed to improve upon the performance of the first-generation TMA technology. All first-generation NAATs including PCR, LCR, SDA and TMA have technical issues that have limited their acceptance in clinical microbiology laboratories. Many of the technical problems associated with the first-generation NAAT tests are due to inadequate and laborious specimen processing steps. Current sample processing procedures are labor-intensive, often requiring centrifugation and organic extraction steps. These methods often do not adequately purify the target nucleic acid and may leave inhibitory or interfering substances in the reaction mixture that can cause inhibition of the amplification reaction resulting in false-negative results [15]. In the case of NAAT tests for CT and GC, it has been reported that the multiple steps of the current sample processing techniques can lead to specimen cross-contamination that can cause false-positive results [16,17]. False-positive results can also occur in some first-generation NAAT tests due to cross-reactivity with other *Neisseria* species [18].

Target capture specimen processing

Gen-Probe's target capture method was designed to overcome many of the technical problems of the first-generation specimen processing methods including previous target capture methods. The target capture procedure works by first lysing the microorganisms to release the target nucleic acid (FIGURE 3). The target sequence is hybridized to an intermediate capture oligomer. This capture oligomer–target complex is then captured by oligo-dT oligomers bound to the surface of 1 µm paramagnetic particles. The particles are drawn to the side of the tube by magnets and washed several times to purify and concentrate targets and remove extraneous material including potentially interfering substances.

Target capture sample processing has been designed to reduce false-negatives by removing the inhibitors and other interfering substances that may occur in clinical samples. Target capture simplifies sample processing and makes automation easier due in part to the absence of centrifugation and other complex steps required in most of the current first-generation NAAT tests. Target capture also concentrates target and allows use of a larger specimen volume. Assay specificity is increased because the nucleic acid targets are purified before amplification.

Once the target capture step is complete, TMA is used to amplify the captured sequences on the surface of the magnetic particles. No elution of the sequences is necessary. The amplification product is then detected by DKA.

DKA

DKA detection technology is based on the hybridization protection assay (HPA) technology used in nearly all of the Gen-Probe NAT assays. DKA uses two different acridinium ester molecules attached to two different nucleic acid probes [19]. One of the acridinium ester molecules has fast light-off kinetics and the other acridinium molecule has slower kinetics. By targeting two different target sequences, assays can be developed to detect two different organisms at the same time (FIGURE 4). Two results can be obtained simultaneously in the same reaction well using one specimen in one reaction. No reflex testing is necessary to identify the individual organisms.

Application of TMA & APTIMA technologies

Detection of M. tuberculosis *in clinical samples with TMA*

The first application of the TMA technology was to the detection of *Mycobacteruim tuberculosis* (Mtb) in clinical samples. Current culture methods typically take 2–8 weeks or more to confirm the presence of Mtb [20]. Acid-fast smears are rapid and simple to perform but are not specific for Mtb and are only about 50–60% sensitive. The amplified *Mycobacterium tuberculosis* direct test (MTD) was developed using TMA technology and was approved by the FDA for use in clinical laboratories in the USA for smear-positive samples in 1995 and for smear-negative samples in 1998. The MTD test is the only amplification test approved by the FDA for use with both smear-positive and smear-negative respiratory specimens. The procedure is simple and takes about 3.5 h to perform after initial decontamination of the specimen.

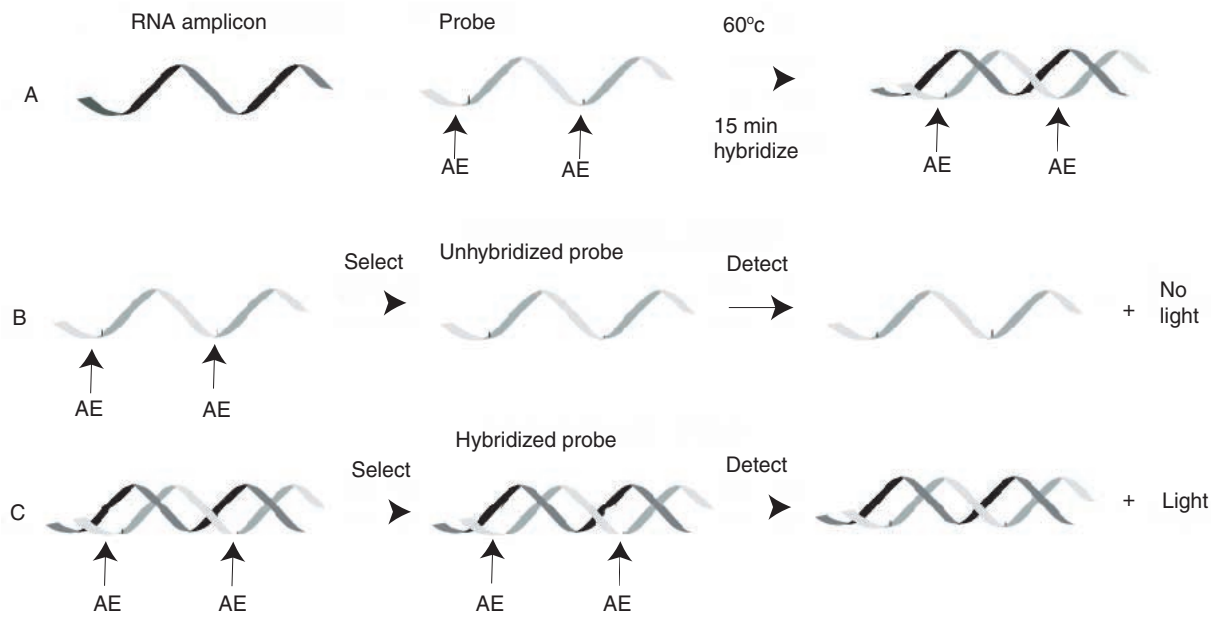

Figure 2. Detection of amplicon with DNA probes and the Hybridization Protection Assay technique. (**A**) Acridinium ester (AE)-labeled DNA probes are added and allowed to hybridize to specific target sequences within the amplicon produced in the transcription-mediated amplification reaction. (**B**) Separation of hybridized from unhybridized probes is done by the addition of selection reagent which hydrolyzes the AE on the unhybridized probes. This effectively separates the hybridized from the unhybridized probe without wash steps or a solid substrate. (**C**) The AE on the hybridized probes is protected within the double helix and is not hydrolyzed by the selection reagent. Light is emitted and detected by a luminometer after addition of detection reagent.

Analytical sensitivity experiments have shown that the MTD test is extremely sensitive and capable of detecting rRNA equivalent to less than one cell of Mtb. Analytical specificity experiments have demonstrated that the MTD test is specific for the members of the Mtb complex which includes *M. africanum, M. bovis, M. microti, M. canetti* and *M. tuberculosis* [21,22].

Many studies have shown that the clinical sensitivity of the MTD test is higher than acid-fast bacillus smear and solid culture and is similar to the sensitivity observed with liquid culture. These studies have also demonstrated high clinical specificity superior to the acid-fast bacillus smear technique [21–30].

The MTD test is useful to the physician for confirming tuberculosis in smear-positive patients so that appropriate isolation measures and antituberculosis treatment can be rapidly initiated. In addition, the MTD test helps the physician distinguish whether a smear-positive patient is infected with Mtb or mycobacteria other than tuberculosis (MOTT). Acid-fast smears do not distinguish between the Mtb complex and MOTT as does the MTD test, which is always negative when only MOTT is present. If a patient is smear-positive and MTD-negative, it is probable that the patient does not have tuberculosis but is infected with MOTT. Further testing can be implemented to verify the MOTT infection. Appropriate treatment decisions can be made more quickly since the treatment for most MOTT infections is different from the treatment for tuberculosis infections. This is particularly important with patients who are infected with HIV. These patients commonly become infected not only with Mtb but also with MOTT, such as *M. avium*. *M. avium* is ubiquitous in the environment but does not normally infect healthy people. Immunocompromised individuals, such as HIV patients can be infected and may rapidly die from the *M. avium* infection if not treated appropriately. The MTD test can quickly rule out the presence of tuberculosis disease in smear-positive, immunocompromised patients leading to more rapid and effective treatment for MOTT infections. This can also result in overall lower healthcare costs because the MTD test can help identify smear-positive patients who are not infected with Mtb and allow the physician to remove those patients from isolation.

The MTD test can also be used to test samples from smear-negative patients. A MTD-positive, smear-negative result will allow earlier, more appropriate treatment of TB-infected patients. Recent studies have suggested that smear-negative, TB-infected patients are capable of transmitting disease [31]. The MTD test can help to control the spread of TB by identifying infected patients in 1 day, rather than the 2–4 weeks required for results from traditional culture methods.

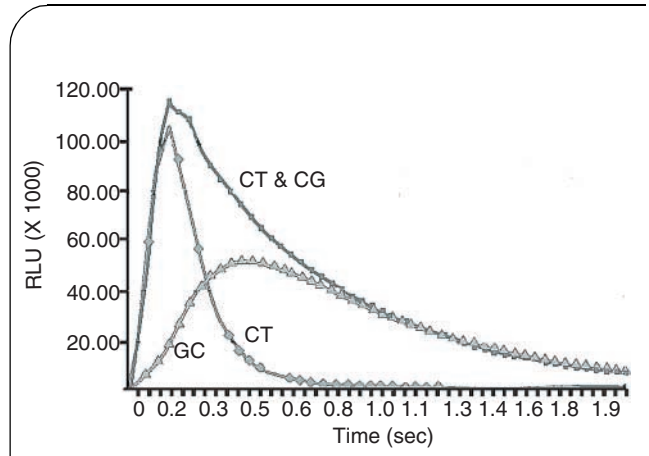

Figure 4. Dual kinetic assay method using the APTIMA Combo 2 *C. trachomatis*/*N. gonorrhoeae* assay as an example. The x-axis is the time interval and the y-axis is the light output measured in RLU. A DNA probe specific for *C. trachomatis* is labeled with a flasher acridinium ester molecule and a very brief flash of light is observed. The probe for *N. gonorrheae* is labeled with a glower molecule and has slower light-off kinetics. If both probes react in the test, an intermediate signal is obtained. The luminometer is able to distinguish between the different signals and determine which organisms are present in the sample.

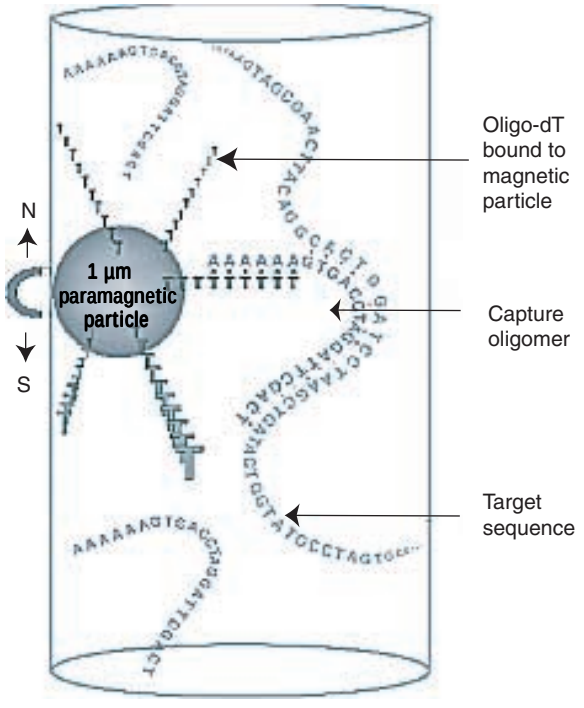

Figure 3. Diagram of the target capture specimen processing method for the Gen-Probe transcription-mediated amplification assays. The target capture procedure works by first lysing open the microorganisms to release the target nucleic acid. The target sequence is then hybridized to an intermediate oligonucleotide capture sequence. This sequence is captured by oligonucleotides bound to the surface of magnetic particles. The magnetic particles are drawn to the side of the test tube by a magnet and the particles are washed several times to remove interfering substances.

Detection of CT & GC in clinical specimens with APTIMA technology

CT and GC are sexually transmitted diseases that typically affect the urethra of males and the endocervix and urethra of females. The diseases, particularly CT, are often asymptomatic or 'silent' but can cause many serious complications in females, such as pelvic inflammatory disease, ectopic pregnancy and sterility [32]. The diseases are easily and inexpensively treated but are not always easy to diagnose due to the lack of clinical symptoms in many infected patients. CT is the most prevalent sexually transmitted disease in the USA and Europe with an estimated 3 million new infections annually in the USA alone. GC is also prevalent in the USA with an estimated 650,000 new infections annually.

Laboratory diagnostic tests for CT and GC are usually designed for use on samples obtained with urethral or endocervical swabs. GC has traditionally been detected by culture and this method is still widely used. The gold standard for CT laboratory diagnostics traditionally has been growth of the organism in cell cultures. However, the cell culture technique is relatively difficult to perform correctly, requires specialized expertise and gives variable results in different laboratories. Direct fluorescence assays (DFA) are potentially sensitive but are labor-intensive and not suitable for large numbers of tests. Enzyme immunoassays for CT are still used but are relatively nonspecific and most of the tests are insensitive compared to culture [33–35]. The PACE 2 nonamplified DNA probe assay for CT has similar performance to culture and is comparable in ease-of-use to the immunoassays [7–12]. The PACE 2 assay has the additional ability to simultaneously identify GC from the same patient sample using a GC-specific probe reagent. Dual detection of both organisms is clinically advantageous because both infections are present in up to 50% of cases [36]. It is important for the physician to be able to distinguish between the two diseases since they have similar symptoms but different treatments.

Despite the availability of several accurate nonculture tests for CT and GC using urogenital swab samples, detection of the organism in urine is desirable because of the noninvasive nature of these samples compared to swab samples. NAAT tests are required for accurate detection of these organisms in urine since CT and GC typically exist in very low numbers in these samples. Several first-generation NAAT tests have been approved by the FDA for use with urine samples including PCR, LCR, TMA and SDA tests. These tests are highly sensitive and are able to detect these organisms in both urine and urogenital swab samples. However, the sensitivity of these tests with female urine samples has been reported to be less than that with endocervical samples, due in part to high inhibition levels observed with these samples [15]. In addition, inconsistent results are sometimes obtained due partly to laborious sample preparation steps, such as centrifugation [37]. The APTIMA Combo 2 (AC2) test was recently developed by Gen-Probe to address many of the limitations of the first-generation NAATs. The AC2 assay was cleared by the FDA in May 2001 for use in the detection of CT and GC in male and female urogenital swabs and urine specimens from both symptomatic and asymptomatic individuals.

The AC2 test uses an identical target capture specimen processing procedure for both urine and urogenital samples with no centrifugation required, which makes the specimen processing easier to perform. The entire procedure has been semiautomated and up to 200 samples can be tested in less than 6 h.

The performance of the AC2 assay was evaluated in a clinical study using urine and swab specimens from 2932 male and female patients at seven clinical sites in the USA [38–42]. AC2 was compared with the results of two other amplification assays, the LCx assay for CT and GC (Abbott Laboratories. Abbott Park, IL, USA) and the AMPLICOR PCR test for CT (Roche Diagnostic Systems, Inc., Branchburg, NJ, USA). GC culture was also used as a comparison test. Results were determined for each test and compared to an infected patient standard. For CT, a positive patient was defined as positive results from any two reference amplification tests. The criterion for GC-infected patient standard was defined as positive GC culture or positive swab and urine amplification results using the reference methods. TABLES 2 and TABLE 3 show the performance characteristics for the amplification tests and GC culture. On a rotating basis, the swab and urine CT results of each of the three amplification tests were compared to an infected patient standard based on the other two assays. Similarly, the swab and urine GC results of each of the two amplification tests were compared to an infected patient standard based on the other

Table 2. Clinical sensitivity and specificity of the AC2 assay compared with two other nucleic acid amplification technology assays for CT infection.

	Swab			Urine		
Women	*Combo 2*	*LCx*	*AMPLICOR*	*Combo 2*	*LCx*	*AMPLICOR*
Sensitivity	94.2	85.6	86.4	95.3	84.1	81.7
Specificity	97.6	99.4	99.1	98.9	99.4	99.4
Men	*Combo 2*	*LCx*	*AMPLICOR*	*Combo 2*	*LCx*	*AMPLICOR*
Sensitivity	96.9	91.1	nd	97.9	89.1	nd
Specificity	97.5	99.2	nd	98.5	99.9	nd

amplification assay and culture. Results from this study show that the AC2 assay was significantly more sensitive than the LCx and PCR assays for both CT and GC. Sensitivity of AC2 for CT using female urine samples was very high (95.3%) and essentially equivalent to that observed for swab samples. This was unusual since sensitivity of amplification assays for urine samples has been reported to be lower than swabs for most first-generation amplification tests due in part to inhibition factors. Specificity of the AC2 test was slightly lower than the other comparator tests. Further studies were performed to analyze the apparent false-positives giving rise to the lower specificity. Apparent false-positive samples were tested by a TMA assay targeting a different rRNA target in the CT and GC organisms. A positive result in the alternate TMA assay suggests that true organism target was present in the sample and the original positive was not due to a carry-over contamination event. TABLE 4 shows that the majority of the apparent false-positive samples were positive in the alternate TMA test. This suggests that many of the apparent AC2 false-positive samples were actually true positives that were undetectable by the comparator amplification and culture tests due to either inhibition or low organism load.

A number of studies were conducted to determine the analytical performance of the assay [42]. The AC2 assay was able to detect CT and GC at levels as low as 0.01 IFU/assay and 50 cells/assay, respectively. No cross-reactivity was detected when testing nontarget organisms and closely related *Neisseria* species.

A recent study has shown that the inhibition rate of the AC2 assay with female urine samples was extremely low when compared to that observed with the LCx amplification test [43]. In this study, CT organisms were spiked into female urine samples obtained from 415 normal women and tested with both the AC2 and LCx assays. The analytical sensitivity of AC2 was more than 100-fold greater than LCx. The inhibition rate for the AC2 and LCx assays was 0.48 and 12.2%, respectively. The authors concluded that the AC2 assay had a higher analytical sensitivity and lower inhibition rate when compared with the LCx assay. These data support the hypothesis that the Gen-Probe target capture specimen processing method is able to greatly reduce or completely eliminate inhibitory factors from specimens.

Blood bank NAAT test for HIV-1 & HCV

Despite improvements in HIV and HCV serological tests in recent years, there are still instances of viral transmission from infected blood donors due to donations while a donor is in the preseroconversion window phase, infection with immuno variant viruses or donation by nonseroconverting chronic carriers. Direct, sensitive detection of viral nucleic acid could substantially decrease the chance of these infections.

Gen-Probe has developed a multiplex NAAT test for the simultaneous detection of HIV-1 and HCV RNA in donated blood samples. This assay was developed under a contract with the National Heart, Lung and Blood Institute for the purpose of decreasing the risk of HIV or HCV transmission *via* transfusion. The Chiron Procleix™ HIV-1/HCV assay uses the same four technologies as the APTIMA Combo 2 tests: target capture, TMA, HPA and DKA. The test was developed and manufactured by Gen-Probe and is distributed to the worldwide blood-screening market by Chiron Blood Testing, a division of Chiron Corporation. The test is currently approved and in use to screen the blood supply in several countries outside the USA and is in Phase III clinical trials at several blood bank sites in the USA using the Procleix system incorporating an automated pipetting station. The procedure is very similar to the AC2 test.

Analytical sensitivity studies have shown that the assay can reliably detect less than 100 copies/ml of HIV-1 or HCV RNA [44]. These same studies have shown that the test can detect all known subtypes of both viruses including HIV-1 Group O and N strains with similar sensitivity.

The American Red Cross (ARC), America's Blood Centers (ABC) and Association of Independent Blood Centers (AIBC) have been conducting Phase II clinical trials with the assay since April 1999 [45]. In the USA, samples from individual donations are pooled into lots of 16 donations each and tested with the Procleix TMA HIV-1/HCV assay in addition to all existing serological tests required. These studies have shown that from a total of over 11,000,000 whole blood donations collected and tested, three HIV and 43 HCV serology-negative samples were identified with the test (TABLE 5). This represents samples that could have been transfused and potentially infected up to 138 people with virus, if the assay had not been implemented (up to three people may be transfused with products from a single blood

Table 3. Clinical sensitivity and specificity of the AC2 assay compared with another nucleic acid amplification technology assay and culture for GC infection.

	Swab				Urine			
	Sensitivity		Specificity		Sensitivity		Specificity	
	Combo 2	*LCx*	*Combo 2*	*LCx*	*Combo 2*	*LCx*	*Combo 2*	*LCx*
Women	99.2	92.4	98.7	99.7	91.1	80.9	99.3	99.9
Men	99.2	97.0	97.9	99.3	98.1	97.4	99.6	99.9

donation). Samples that were positive for virus by the Procleix assay but negative by antibody assays were confirmed by tests using an alternative NAAT test or by follow-up testing of the donor [46]. Specificity was greater than 99.95% at all sites.

The Procleix TMA HIV-1/HCV assay incorporates an internal control to monitor for inhibition of the amplification reaction. The Phase II studies have demonstrated an initial internal control failure rate of less than 0.8% and a 0% failure rate upon repeat testing [44]. This again provides evidence that the Gen-Probe target capture method virtually eliminates inhibition in the assay.

These studies show that the Procleix TMA HIV-1/HCV assay has a significant sensitivity advantage over the existing serological tests for the detection of viremic donated blood samples. Preliminary data indicates that HIV-1 can be detected with the Procleix HIV-1/HCV assay an average of 16.3 days before antibody detection and 7.5 days before detection using P-24 antigen testing [44]. Use of the TMA assay expedites detection of HCV an average of 32.8 days before antibody detection. Implementation of the assay in routine blood bank testing will achieve the FDA and NIH goal of using the latest technology to continue to improve the safety of the USA blood supply. The high volume of donated samples processed through blood bank laboratories necessitates the use of efficient, automated testing. The ability of the Procleix TMA HIV-1/HCV assay to detect both organisms at the same time, together with the eventual implementation of full automation, will allow the blood bank laboratories to efficiently use the more sensitive and specific TMA test with donated blood samples.

The Procleix TMA HIV-1/HCV assay has not yet been licensed for use in the USA but is already used with 70% of blood donations in the USA under an investigational new drug application. The assay is currently in commercial use in France, Italy, Portugal, Spain, Australia and Singapore.

Table 4. Transcription-mediated amplification alternate amplification testing results of samples that were AC2 positive and negative in the nucleic acid amplification technology comparator tests.

Sample type	LCx CT-negative		PCR CT-negative	
	Alternate Amp. tested	*Alternate Amp. positive*	*Alternate Amp. tested*	*Alternate Amp. positive*
Male swab	25	20	28	19
Male urine	36	26	40	33
Female swab	41	34	30	19
Female urine	26	16	30	19
Total	128	96	128	90

Table 5. Results of blood screening using the Procleix transcription-mediated amplification HIV-1/HCV assay among blood donors in the USA.

Test sites	Units tested	HCV-reactive units	HIV-1-reactive units
American Red Cross	7.7 million	25 (1:308,000)	1 (1:7.7 million)
America's Blood Centers	2.96 million	16 (1:185,000)	2 (1:1.5 million)
Association of Independent Blood Centers	375,000	2 (1:187,500)	0

Summary & expert opinion

Nucleic acid probe and NAAT technologies have become widely established in the clinical microbiology laboratory in recent years. The clinical laboratory acceptance of these tests has been driven by the development of more accurate and less labor-intensive molecular diagnostic tests. This is exemplified by Gen-Probe's introduction of the rRNA targeting and HPA technologies in the late 1980s, which were a significant improvement over the existing home-brew nucleic acid probe assays. A parallel development has recently occurred with the NAAT tests. An example of this is the evolution of the TMA technology to the recently developed APTIMA technology that simplifies and improves the specimen processing technology and allows the simultaneous multiplex detection of two organisms.

The APTIMA Combo 2 test for CT and GC has been shown to be more sensitive than both culture and two currently available NAAT tests. This higher sensitivity is due to a combination of rRNA targeting, the high analytical sensitivity of TMA and the virtual elimination of inhibition using Gen-Probe's target capture technology. Similarly, the Procleix TMA HIV-1/HCV assay has also been shown to be extremely sensitive due in part to the lack of inhibition as shown by the internal control data. This assay has become extremely important in screening donated blood worldwide.

Full automation will be the next step needed for molecular diagnostic testing. All of the current nucleic acid probe and NAAT tests are either completely manual or semiautomated at best. Full automation will not only decrease costs through a reduction in labor needs but will also minimize operator error and maximize performance leading to a more widespread acceptance of NAAT testing in clinical laboratories.

Five-year view

Over the next 5 years, assays using the HPA, TMA and APTIMA technologies will become increasingly automated, which will make these assays more widely available to clinical laboratories. These technologies will also be applied to a larger array of infectious disease organisms and to the development of additional clinical laboratory tests in fields, such as cancer and genetic testing.

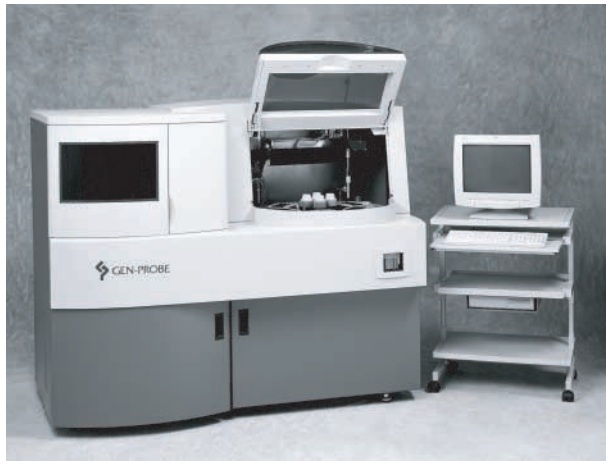

Figure 5. The TIGRIS instrument fully automates all steps of the transcription-mediated amplification tests.

Full assay automation is needed to overcome many of the problems associated with the first-generation NAAT tests [47]. Larger clinical laboratories are particularly hindered from adopting molecular diagnostic technologies because of the high labor requirements and low throughput of many of the first-generation NAAT testing systems. Gen-Probe is developing the first fully automated instrument for NAAT testing. The TIGRIS™ instrument system (FIGURE 5) has been designed to automate all steps of the testing procedure, from sample processing through amplification and detection. The throughput goal of the instrument is up to 500 tests per 8 h shift or 1000 tests per 12 h shift. It takes about 3.5 h to obtain the first result and up to 125 sample results can be obtained each subsequent hour depending on the particular assay. The only sample preparation required is to load the specimens directly on board the system. With minimal sample handling, the risk of technologist errors resulting in cross-contamination should be significantly reduced. The TIGRIS instrument also automatically destroys amplicon on board to help decrease the chance of carry-over contamination. Specimen bar code reading, automatic worklist creation from the input carousel and bi-directional communication with a Laboratory Information System will be an integral part of the TIGRIS instrument, allowing positive sample identification and increasing overall NAAT testing efficiency. The first assays that will appear on the TIGRIS instrument will be the AC2 assay for CT and GC and the Procleix TMA HIV-1/HCV assay. The TIGRIS instrument will allow many larger clinical laboratories to adopt NAAT testing more easily by decreasing the labor requirements and thus the overall costs of this type of testing.

TMA tests utilizing target capture have recently been developed for *C. pneumoniae*, *Mycoplasma pneumoniae* and *M. tuberculosis*. A triplex test simultaneously targeting HIV-1, HCV and HBV in the same sample using these technologies is being developed as a potential test for the blood bank. Quantitative TMA tests are also currently under development for both HIV and HCV. A TMA test for chronic myelogenous leukemia is currently available in an assay-specific reagent format. Several other TMA tests are being developed in the cancer and genetic fields. Some of these future assays will be implemented on the TIGRIS instrument for those tests requiring larger volume, automated testing.

Nucleic acid probes and amplification technology are undergoing an exciting and extremely rapid pace of innovation and change. New developments will continue to improve upon the performance, laboratory efficiency, throughput and cost-efficiency of the tests. New infectious disease and cancer analytes are being targeted for development of diagnostic assays that will far surpass many of the currently available tests in speed and performance. Nucleic acid technology will continue to replace many of the current, more time-consuming diagnostic assays, ultimately resulting in more accurate and timely patient diagnosis.

Key issues

- Commercial assay kits using DNA probe and nucleic acid amplification technologies for infectious disease detection have been made simple and accurate enough to be used in clinical microbiology laboratories.
- Recent developments in transcription-mediated amplification technology using target capture technology have improved sample processing to increase the accuracy and laboratory efficiency of the transcription-mediated amplification assays by virtually eliminating specimen inhibition that is often observed with nucleic acid amplification technology tests. These tests also allow simultaneous detection of two organisms at the same time in the same reaction tube using dual kinetic assay technology.
- Detection of *M. tuberculosis* using nucleic acid amplification technology tests, such as the MTD assay has improved the accuracy and turnaround time of TB laboratory diagnostics.
- Nucleic acid amplification technology tests, such as the APTIMA Combo 2, increase the accuracy for detecting *C. trachomatis* and *N. gonorrhoeae* in clinical samples and allows the use of noninvasive samples such as urine.
- Instituting the transcription-mediated amplification test for the detection of HIV-1 and HCV in blood bank samples has led to the detection of additional infected samples that would have been missed by existing detection methods.
- Nucleic acid amplification technology tests need to be automated to increase the laboratory efficiency and decrease the cost of these tests.
- Development is progressing to fully automate transcription-mediated amplification tests on the TIGRIS instrument.

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