McDonough, S H., Bott, M., & Giachetti, C. (1997) in *Molecular and Laboratory Medicine Series: Nucleic acid amplification technologies: Application to disease diagnosis* (Lee, H.H., Morse, S.A., & Olsvik, Ø., Ed.) pp 113–123, Eaton Publishing Co., Natick, MA.

Application of Transcription-Mediated Amplification to Detection of Nucleic Acids from Clinically Relevant Organisms

Sherrol H. McDonough, Martha A. Bott and Cristina Giachetti

Gen-Probe Inc., San Diego. CA. USA

INTRODUCTION

Detection and identification of infectious organisms are important aspects of diagnosis of infectious disease. Traditionally, culture is performed to allow replication of the suspected organism to a detectable level and identification is achieved by subsequent biochemical characterization. Although culture requires hours to days to complete, it is often considered the gold standard for infectious agent identification. In some instances, diagnosis is accomplished by detection of the host immune response to the suspected infectious agent, provided that exposure is rare among the general population and that time is allowed for development of an immune response. Nucleic acid-based diagnostic assays combine the advantages of direct organism detection and identification with the rapid turnaround time and convenience of antibody-based detection methods. Such assays have proven useful for detection of infectious agents that are difficult or impossible to identify with standard antibody- or culture-based methods.

DNA probe-based assays are currently used in clinical laboratories for confirmation of diagnosis and for screening of potential infections in certain populations, such as patients attending sexually transmitted disease clinics (13,19,30). For applications requiring additional sensitivity, specific amplification of the nucleic acid of interest of the hybridization signal is used. Ligase chain reaction (LCR), polymerase chain reaction (PCR), strand displacement amplification (SDA), nucleic acid-based amplification (NASBA) and transcription-mediated amplification (TMA) represent approaches that have been applied to specifically amplify nucleic acids from clinically relevant organisms and genetic markers for disease (13,15).

While identification of an infectious agent may be sufficient for diagnosis, assessment of patient prognosis may depend upon the number of organisms present. Thus adaptation of probe and amplification assays to a quantitative mode expands the utility of the technology. Quantification of cells bearing a

particular genetic marker may be used as an indication of clinical progression in leukemias or other cancers (10,12,23). Determination of pathogen load or quantification of cells bearing a particular genetic marker is also useful for assessment of therapeutic efficacy. Individualizing treatment based on neoplastic load is common for cancer patients, and individualization of treatment of patients with infectious diseases, particularly patients with chronic infections, is expected to become more common.

The utility of probe-based testing is increased by combining probes to assay for more than one infectious agent at a time. Such an approach is especially useful for screening, where cost issues are significant and most specimens are expected to give negative results. Combining probes may also facilitate diagnosis in medical situations that require immediate identification of organisms, such as meningitis or pneumonia. Concurrent analysis of multiple targets can also be applied to detect nucleic acid sequences responsible for drug resistance and to screening for disease susceptibility, where more than one genetic change may have to be identified.

In this chapter, we describe an amplification method for detection of infectious agents in clinical specimens. Examples of assays that allow quantification of viral nucleic acid or the simultaneous detection of two organisms in patient specimens are also described.

TMA ASSAYS

Assays based on TMA consist of three basic steps: sample processing, nucleic acid amplification and specific detection. During these steps, nucleic acid is made available for assay, amplified to a detectable level and identified by hybridization to a specific oligonucleotide probe. The sample processing step is responsible for lysing the organisms or cells of interest, releasing and stabilizing nucleic acids and minimizing or removing potential assay inhibitors or interfering factors. Chemical lysis has been used for the examples in this chapter. The overall sensitivity, accuracy and reproducibility of the test results are affected by the quality of sample processing. In assays with stringent sensitivity requirements, concentration of the organism by centrifugation or concentration of the nucleic acid itself has been performed (see below).

The amplification step serves to increase the number of copies of RNA or DNA to a detectable level. A greater than one billion-fold increase in copy number of the targeted sequence is achieved with the use of two enzymes, reverse transcriptase and RNA polymerase. The enzymes accomplish the amplification in 1 h or less at one incubation temperature, typically 42°C. Because no thermocycling is required, TMA is readily adapted for automation. Examples of nucleic acids amplified by TMA include highly structured ribosomal RNA (rRNA), cellular messenger RNA, viral RNA and viral DNA.

Figure 1 shows a TMA reaction scheme for an RNA target. In this reaction, the input RNA is converted to a double-stranded DNA intermediate by reverse transcriptase in the presence of a sense primer and an antisense primer with an

RNA polymerase-specific promoter sequence at the 5′ end. The DNA intermediate thus contains a double-stranded promoter sequence that is specifically recognized by RNA polymerase and is transcribed into hundreds of copies of RNA. Each RNA molecule can be converted into a new double-stranded DNA intermediate, which produces more RNA, allowing the reaction to proceed exponentially. Thus, in a few cycles, a greater than 10^9-fold amplification of the original target is achieved. For a detection system with sensitivity of 10 fmole, 10^9-fold amplification is sufficient to allow detection of 10 copies of input target. For multiple-copy sequences such as rRNA or plasmids, the amplification

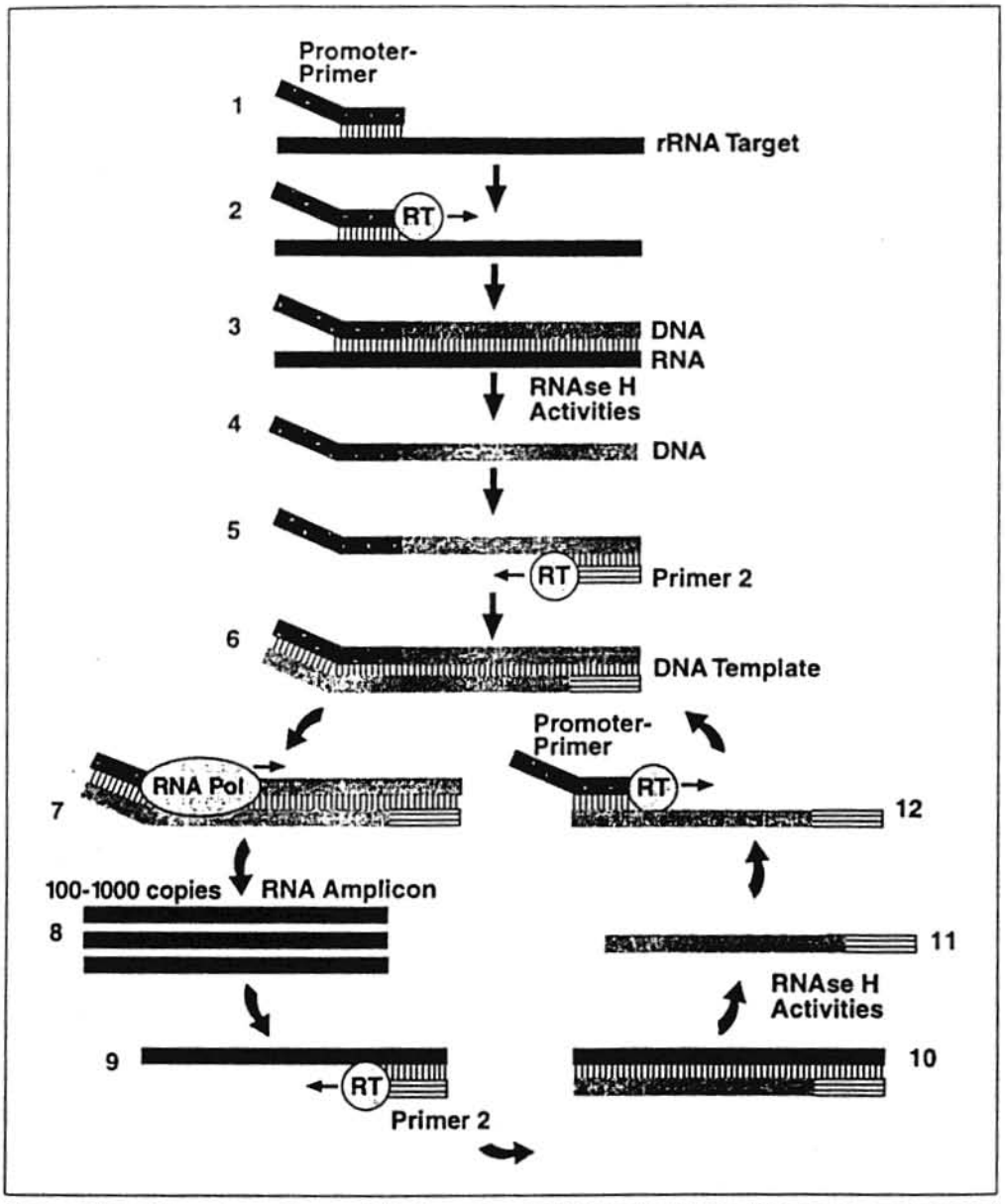

Figure 1. Reaction scheme for amplification of an RNA target by TMA. RT, reverse transcriptase; RNA Pol, RNA polymerase.

efficiency of TMA is sufficient to detect less than one cell equivalent of RNA or DNA.

Detection of RNA amplicons produced by the TMA reaction has been achieved with a system known as the hybridization protection assay (HPA). HPA relies on target-specific oligonucleotide probes coupled to the chemiluminescent label acridinium ester and, when performed in the presence of excess probe, yields quantitative results. After hybridization, typically performed for 15 to 30 min at 60°C, an alkaline selection reagent is added. Under alkaline conditions, acridinium ester associated with unhybridized probe is hydrolyzed to a nonchemiluminescent form. Label associated with hybridized probe is protected from hydrolysis and, therefore, is chemiluminescent when exposed to appropriate detection reagents in a luminometer. Results from this assay are given in relative light units (RLU), and are a direct measure of light produced in the luminometer, which in turn is a direct measure of the amount of hybrid formed. HPA has a linear dynamic range of greater than three log units and is sufficiently sensitive to allow for detection of less than 10 copies of the original target molecule in the clinical specimen, assuming an amplification factor of 10^9 (24).

A recent modification of this assay allows for the simultaneous detection of more than one nucleic acid (22). The dual detection system relies on modified acridinium ester labels with different chemiluminescence kinetics.

Detection of *Chlamydia trachomatis* Based on rRNA Target

In some situations, detection of an infectious organism in a particular clinical sample is sufficient to make a diagnosis. One such example is the detection of the intracellular bacterium *C. trachomatis*. This organism is responsible for infections of the genital-urinary tract as well as conjunctivitis and pneumonia (29). It is also the causative agent of the eye disease trachoma (28). If left untreated, individuals infected with *C. trachomatis* may develop complications such as pelvic inflammatory disease, infertility and ectopic pregnancy in women, or epididymitis in men. Infections with *C. trachomatis* are a major cause of sexually transmitted disease, with more than 4 million new cases of chlamydial infection occurring annually in the United States and 50 million worldwide (8). A large portion of infections may be asymptomatic or have symptoms that are not specific. Accurate and prompt diagnosis of these infections is important to ensure appropriate patient management, to prevent disease complications and their associated medical costs and to control transmission to uninfected partners.

Amplification assays based on LCR or PCR have been described for detection of *C. trachomatis* (4,17,25,26,31). We have applied TMA and HPA technology to the detection of *C. trachomatis* in genital swabs and urine. Detection of this bacterium is facilitated by the fact that each elementary body contains thousands of copies of rRNA, and that sequences exist in the rRNA that are specific for *C. trachomatis*. We therefore targeted these sequences for amplification primer and detection probe design. Chlamydia cells were lysed directly in

Table 1. Sensitivity of Detection of *C. trachomatis* rRNA by TMA and HPA

Number of rRNA copies	RLU* in Buffer	RLU in Swab Specimen	RLU in Urine Specimen
2000 (5 fg)	2224434	Not done	Not done
200 (0.5 fg)	2579673	2458521	2134102
100 (0.25 fg)	Not done	2468335	2206480
40 (0.1 fg)	Not done	2468984	2106991
20 (0.05 fg)	1852421	2036241	2139554
0	4495	4159	4432

The indicated amounts of rRNA were spiked into specimen transport buffer or genital swab or urine specimens and then assayed in triplicate or quadruplicate.

*Values expressed as average of replicates

swab specimen using detergents. Chlamydia cells in urine were pelleted and then processed prior to amplification.

The limit of detection in terms of rRNA copies was determined by serially diluting a purified stock of *C. trachomatis* rRNA in specimen transport buffer or in swab or urine specimens from uninfected individuals. The assay (Amplified Chlamydia Trachomatis Assay, or AMP CT) is capable of reliably detecting as few as 20 copies (0.05 fg) of rRNA (Table 1), which is equivalent to 1% of the rRNA in a single *C. trachomatis* cell. The limit of detection of the assay was also determined by amplifying known quantities of cultured *C. trachomatis* cells representing all 15 serovars. Each cell stock was quantified in terms of inclusion forming units (IFU) in culture and dilutions of the cell stocks were amplified in the AMP CT assay. The data for the 15 serovars were similar to the results obtained with purified rRNA, yielding saturated signals with less than one IFU equivalent of *C. trachomatis* (data not shown).

The assay is specific for *C. trachomatis* and does not show cross-reaction with any of 117 culture isolates evaluated. These isolates included 73 organisms that may be detected in the urogenital tract and 44 additional organisms that represent a phylogenetic cross-section. Representative bacteria, fungi, yeast, parasites and viruses were tested, as were *Chlamydia psittaci* and *Chlamydia pneumoniae*. The bacterial and fungal organisms were tested at concentrations of 2.5×10^5 cells per assay; five organisms were tested with purified rRNA (2.4 to 23.8 ng per assay) because cell lysates were not available. Only the *C. trachomatis* samples produced positive results. Other organisms gave values ranging from 3485 to 11406 RLU, all of which were well below the assay cutoff of ≥50000 RLU.

The assay was evaluated in a clinical study of 2295 subjects (1510 women

Table 2. Summary of Clinical Evaluation of the AMP CT Assay

Sample type	Sensitivity (%)	Specificity (%)
Swabs		
Endocervical	99.2 (121/122)	99.8 (1372/1375)
Male urethral	98.6 (71/72)	99.7 (327/328)
Urine		
Female	84.8 (95/112)	99.8 (1258/1261)
Male	94.5 (120/127)	99.5 (636/639)

and 785 men) at five geographically distributed sites in the United States. These sites represented female populations with a high or low prevalence of infection with *C. trachomatis* as well both symptomatic and asymptomatic men and women. The prevalence of *C. trachomatis* infection determined by culture ranged from 4.0% to 13.6%, with an overall prevalence of 6.6%. Culture results from endocervical swabs served as the reference to which AMP CT endocervical and female urine results were compared. Culture results from urethral swabs served as the reference for AMP CT male urethral and urine results. For this study, an expanded reference standard was used to determine AMP CT assay performance. Samples positive by cell culture were considered positive. Apparent AMP CT false-positive (culture-negative, AMP CT-positive) specimens were tested with direct fluorescent antibody staining and with a TMA assay targeting an alternative rRNA molecule. The alternative TMA assay discriminated between true target rRNA amplification and contamination by amplicon from the primary AMP CT assay. The data were analyzed with an assigned cutoff together with an equivocal zone. Samples yielding results within the equivocal zone were retested and considered positive or negative based on the assigned cutoff value. Samples yielding initial results outside of the equivocal zone were considered positive or negative as appropriate. The overall study results for each sample type are shown in Table 2.

These data indicate that TMA is capable of detecting the infectious organism *C. trachomatis* directly in clinical specimens with high sensitivity and specificity.

Quantitative Detection of Viral Nucleic Acids with TMA

Adaptation of sample processing, signal or target amplification, and detection steps to a quantitative mode expands the possible applications of amplification technology (7,20,32). One such application is the analysis of viral load to assess patient prognosis and treatment efficacy. The example shown in Figure 2 demonstrates the ability of

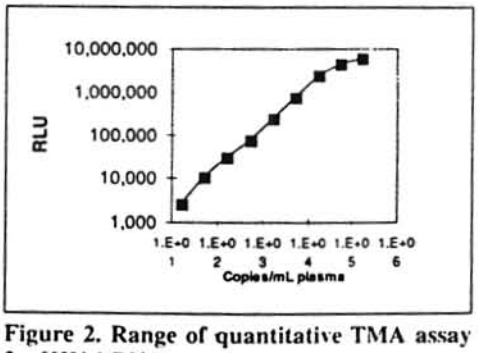

Figure 2. Range of quantitative TMA assay for HIV-1 RNA.

TMA and HPA to quantify the amount of human immunodeficiency virus-type 1 (HIV-1) RNA in plasma. HIV-1 virions are detected in plasma soon after infection, and the concentration of viral RNA correlates with the probability of disease progression (11,16). Recent antiretroviral therapies have been shown to reduce the concentration of viral RNA in plasma. The assay we have developed for HIV-1 RNA in plasma includes treatment with a detergent to lyse the virus and inactivate nucleases, an RNA isolation step, quantitative amplification with TMA, and quantitative detection by HPA. All steps of this assay are performed in the same reaction tube, which improves precision by minimizing pipetting steps and also minimizes carryover contamination. The results for reactions containing virus representing from 16 to 160 000 copies of viral RNA per milliliter of plasma are shown (Figure 2). The utility of the assay for patient monitoring was studied by assaying specimens from HIV-1-infected individuals undergoing treatment with dipyridodiazepinone, a non-nucleoside reverse transcriptase inhibitor. Plasma samples were stored and analyzed retrospectively. The assay detected changes in RNA concentration after initiation of therapy (Figure 3). The same assay showed no change in RNA concentration in an untreated individual over four time points (10).

A DNA virus, hepatitis B virus (HBV), has also been quantified using TMA and HPA. An assay for HBV DNA with a dynamic range of more than four log units (from 10^2 to greater than 10^6 copies/mL has been described (10). The assay was useful for monitoring patient response to interferon therapy, and the concentration of viral DNA in plasma was shown to parallel that of another viral marker, hepatitis B e antigen.

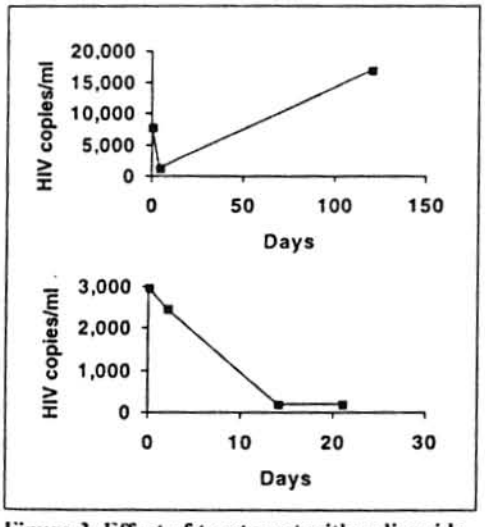

Figure 3. Effect of treatment with a dipyridodiazepinone on the number of HIV-1 RNA molecules in the plasma of two patients infected with HIV-1. Plasma specimens were analyzed by TMA and HPA. Samples were kindly provided by Dr. Doug Richman of the University of California, San Diego, CA, USA.

Simultaneous Detection of Two Analytes in One Tube

Sample preparation, amplification and detection steps of TMA-based assays can be adapted to detect more than one target nucleic acid simultaneously. Sample processing can be readily modified to handle more than one target at a time and the next example demonstrates the ability of TMA to amplify two unrelated RNA molecules simultaneously. Detection can be performed by hybridizing with a mixture of two probes or by separating portions of the amplification reaction mixture and detecting analytes individually.

One proposed use for such a test is the simultaneous detection of HIV-1 and hepatitis C virus (HCV) for the

Table 3. Simultaneous Amplification of Different Levels of HIV-1 and HCV RNAs in the Same Reaction Tube

HIV-1 RNA copy number	HIV-1 RNA alone	HIV-1 RNA + 10^4 copies of HCV RNA	
	HIV-1 signal*	HIV-1 signal	HCV signal*
0	6678	5250	Not done
10^1	1 788 011	2 034 773	1 145 040
10^2	2 065 001	2 026 035	1 087 285
10^3	2 131 809	2 133 520	1 063 233
10^4	2 245 603	2 251 005	1 108 596
10^5	2 237 804	2 283 857	1 145 291
HCV RNA copy number	**HCV RNA alone**	**HCV RNA + 10^4 copies HIV-1 RNA**	
	HCV signal	HCV signal	HIV-1 signal
0	2420	2230	not done
10^1	135 433	57 520	2 229 235
10^2	661 148	275 217	2 335 614
10^3	1 506 695	1 076 219	2 304 277
10^4	1 745 562	1 572 154	2 157 900
10^5	1 673 223	1 759 005	2 279 759

*Signal expressed as RLU

screening of donated blood. Both viruses are transmitted by exposure to infected blood or blood products, or to certain body fluids or tissues as well as from mother to fetus or child. Current detection of HIV-1 or HCV infection in the blood bank setting is based on serological screening for virus-specific antibodies or HIV-1 p24 antigen. It is estimated that the addition of a sensitive assay for viral RNA would reduce the earliest time at which infection can be detected to 11 days after exposure for HIV-1, and to 1–2 weeks for HCV, thus preventing more than half of the HIV-1 and HCV infections that are caused by blood transfusion (5,6,27). In addition, the use of nucleic acid-based tests would overcome some of the disadvantages of antibody-based tests, such as the inability to differentiate between active and resolved HCV infections, and the false-positive results given by patients with hypergammaglobulinemia or other forms of chronic hepatitis (1,9). They could also aid in the confirmation of infection in HIV-1 or HCV seropositive newborns or in individuals with indeterminate serology.

Multiplex detection of both viruses with one nucleic acid-based test should provide a highly sensitive and cost-effective means to screen for these two analytes. Incorporation of such a test into the routine testing of samples by blood banks also has the potential to reduce reporting errors due to sample handling (20). Co-amplification and detection of HIV-1 and HCV has been described (21) but the format was not designed for high throughput applications. We

Table 4. Simultaneous Sample Processing and Amplification of RNA from HIV-1 and HCV Virions in Plasma

10^5 copies of HIV-1	10^5 copies of HCV + 10^5 copies of HIV-1		10^5 copies of HCV
HIV-1 signal*	HIV-1 signal	HCV signal	HCV signal
1 199 103	1 227 964	2 498 393	2 703 737
10^2 copies of HIV-1	10^2 copies of HCV + 10^2 copies of HIV-1		10^2 copies of HCV
HIV-1 signal	HIV-1 signal	HCV signal	HCV signal
825 966	529 977	160 504	209 219

*Signal expressed as RLU

developed an assay in which sample preparation, amplification and detection were performed in the same reaction tube in a format adaptable to automation for high throughput testing. Conditions for all three steps were optimized for detection of each target virus in the presence of the other virus.

To demonstrate co-amplification of HIV-1 and HCV RNAs, we amplified each transcript alone or in the presence of 10 000 copies of the other target. At the end of the amplification reaction, each reaction mixture was divided and a portion of each reaction was assayed for HIV-1 or HCV amplicon by hybridization with specific HIV-1 or HCV probes. Each condition was tested in triplicate, and mean RLU values for HIV-1 or HCV signals are shown in Table 3. Under optimized conditions, the efficiency of amplification of different amounts of HIV-1 RNA (10 to 10^5 copies) was not affected by the presence of 10^4 copies of HCV RNA as well as HCV primers and probes. Similarly, the amplification of different amounts of HCV RNA (10 to 10^5 copies) was not markedly affected by the presence of 10^4 copies of HIV-1 RNA as well as HIV-1 primers and probes.

HIV-1 and HCV virions were also spiked into normal plasma, processed together and amplified together. Table 4 shows the results obtained after co-processing and co-amplification of 100 or 100 000 HIV-1 and HCV virions in 100 µL of plasma. For comparison, reactions containing only one target were also performed. At the end of the amplification reaction, each reaction mixture was divided in two and each half was assayed for HIV-1 or HCV amplicons by HPA with specific HIV-1 or HCV probes. Each condition was tested with six replicates and mean RLU values for HIV-1 or HCV signals are provided in Table 4. Signals in the absence of targets were less than 10 000 RLU. With either virion, the signal obtained when both viruses were present was similar to that obtained in the presence of only one virus.

The data presented here show the feasibility of simultaneous processing and amplification of two unrelated RNA targets. As requirements for detection of more than two analytes arise, the technologies described can be further adapted to allow processing of many analytes simultaneously.

CONCLUSIONS

The detection of nucleic acids has many important applications. High sensitivity and specificity of the TMA and HPA technologies have been demonstrated in a patient laboratory setting for the detection of *C. trachomatis* rRNA in clinical specimens. Adaptation of TMA and HPA to a quantitative mode has been demonstrated with HIV-1 RNA and HBV DNA targets in plasma with a dynamic range of greater than three log units. In addition, concurrent amplification of two viral targets, HIV-1 and HCV, in the same reaction vessel has been demonstrated. The sensitivity, specificity and versatility of these technologies allow adaptation for many applications.

REFERENCES

1. **Alter, H., R.H. Purcell, J.W. Shih, J.C. Melpolder, M. Houghton, Q.-L. Choo and G. Kuo.** 1989. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N. Engl. J. Med. *321*:1494-1500.
2. **Arnold, L.J., P.W. Hammond, W.A. Wiese and N.C. Nelson.** 1989. Assay formats involving acridinium-ester-labeled DNA probes. Clin. Chem. *35*:1588-1594.
3. **Baron, E.J., L.R. Peterson and S.M. Finegold (Eds.).** 1994. Bailey and Scott's Diagnostic Microbiology. 9th ed. CV Mosby, St. Louis.
4. **Buimer, M., G.J.J. Van Doornum, S. Ching, P.G.H. Peerbooms, P.K. Plier, D. Ram and H.H. Lee.** 1996. Detection of Chlamydia trachomatis and Neisseria gonorrhoeae by ligase chain reaction-based assays with clinical specimens from various sites: implications for diagnostic testing and screening. J. Clin. Microbiol. *34*:2395-2400.
5. **Busch, M.P.** 1995. Applications of molecular biology to infectious disease screening of blood donors, p. 111-138. *In* R. Allen and J. AuBuchon (Eds.), Molecular Genetics in Diagnosis and Research. American Association of Blood Banks, Bethesda.
6. **Busch, M.P., L.L.L. Lee, G.A. Satten, D.R. Henrard, H. Farzadegan, K.E. Nelson, S. Read, R.Y. Dodd and L.R. Petersen.** 1994. Time course of detection of viral and serologic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. Transfusion *35*:91-97.
7. **Cao, Y., D.D. Ho, J. Todd, R. Kokka, M. Urdea, J.D. Lifson, M. Piatak, Jr., S. Chen, B.H. Hahn, M.S. Saag and G.M. Shaw.** 1995. Clinical evaluation of branched DNA signal amplification for quantifying HIV type 1 in human plasma. AIDS Res. Hum. Retroviruses *11*:353-361.
8. **Centers for Disease Control and Prevention.** 1993. Recommendations for the prevention and management of Chlamydia trachomatis infections. MMWR *42* (RR-12):1-39.
9. **Fried, M.W., J.O. Draguesku, M. Shindo, L.H. Simpson, S.M. Banks, J.H. Hoofnagle and A.M. DiBiscelge.** 1993. Clinical and serological differentiation of autoimmune and hepatitis C virus-related chronic hepatitis. Digest. Dis. Sci. *38*:631-636.
10. **Gonzales, F. and S.H. McDonough.** Application of transcription-mediated amplification to quantifiation of gene sequences. *In* F. Ferre (Ed.), Gene Quantification. Birkhauser, Boston (In press).
11. **Ho, D.D.** 1996. Viral counts count in HIV infection. Science *272*:1124-1125.
12. **Hochhaus, A., F. Lin, A. Reiter, H. Skladny, P.J. Mason, F. van Rhee, P.C.A. Shepherd, N.C. Allan, R. Helhmann, J.M. Goldman and N.C.P. Cross.** 1996. Quantification of residual disease in chronic myelogenous leukemia patients on interferon-α therapy gby competitive polymerase chain reaction. Blood *87*:1549-1555.
13. **Hunt, J.M. and D.H. Persing.** 1993. Bacterial detection, p. 525-564. *In* G.H. Keller and M.M. Manak (Eds.), DNA Probes. Stockton Press, New York.
14. **Jonas, V., M.J. Alden, J.I. Curry, K. Kamisango, C.A. Knott, R. Lankford, J.M. Wolfe and D.F. Moore.** 1993. Detection and identification of *Mycobacterium tuberculosis* directly

from sputum sediments by amplification of rRNA. J. Clin. Microbiol. *31*:2410-2416.

15. **Kacian, D.L. and T.J. Fultz.** 1995. Nucleic acid sequence amplification methods. US Patent. 5,399,491.

16. **Kappes, J.C., M.S. Saag, G.M. Shaw, G.H. Hahn, P. Chopray, S. Chen, E.A. Emini, R. McFarland, L.C. Yang, M. Piatak, Jr. and J.D. Lifson.** 1995. Assessment of antiretroviral therapy by plasma viral load testing: standard and ICD HIV-1 p24 antigen and viral RNA (QC-PCR) assays compared. J. Acquir. Immune Defic. Syndr. *10*:139-149.

17. **LeBar, W.D.** 1996. Keeping up with new technology: new approaches to diagnosis of Chlamydia infection. Clin. Chem. *42*:809-812.

18. **LePont, F., D. Costagliolo, C. Rouzioux and A.J. Valleron.** 1995. How much would the safety of blood transfusion be improved by including p24 antigen in the battery of tests. Transfusion *35*:542-547.

19. **Limberger, R.J., R. Biega, A. Evancoe, L. McCarthy, L. Slivienski and M. Kirkwood.** 1992. Evaluation of culture and the Gen-Probe PACE 2 assay for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in endocervical specimens transported to a state health laboratory. J. Clin. Microbiol. *30*:1162-1166.

20. **Mulder, J., N. McKinney, C. Christopherson, J. Sninsky, L. Greenfield and S. Kwok.** 1994. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. J. Clin. Microbiol. *32*:292-300.

21. **Nedjar, S., R.M. Biswas and I.K. Hewlitt.** 1991. Co-amplification of specific sequences of HCV and HIV-1 genomes by using the polymerase chain reaction assay: a potential tool for the simultaneous detection of HCV and HIV-1. J. Virol. Methods *35*:297-304.

22. **Nelson, N.C., A. BenCheikh, E. Matsuda and M. Becker.** 1996. Simultaneous detection of multiple nucleic acid targets in a homogeneous format. Biochemistry *35*:8429-8438.

23. **Nguyen, N.J. and R.A. McPherson.** 1993. Cancer diagnosis, p. 483-523. *In* G.H. Keller and M.M. Manak (Eds.), DNA Probes, 2nd ed. Stockton Press, New York.

24. **Ou, C.-Y., S.H. McDonough, D. Cabanas, T.B. Ryder, M. Harper, J. Moore and G. Schochetman.** 1990. Rapid and quantitative detection of enzymatically amplified HIV-1 DNA using chemiluminescent oligonucleotide probes. AIDS Res. Hum. Retroviruses 6:1323-1329.

25. **Quinn, T.C., L. Welsh, A. Lentz, K. Crotchfelt, J. Zenilman, J. Newhall and C. Gaydos.** 1996. Diagnosis by AMPLICOR PCR of *Chlamydia trachomatis* infection in urine samples from women and men attending sexually transmitted disease clinics. J. Clin. Microbiol. *34*:1401-1406.

26. **Ridgway, G.L., G. Mumtaz, A.J. Robinson, M. Franchini, C. Carder, J. Burczak and H. Lee.** 1996. Comparison of the ligase chain reaction with cell culture for the diagnosis of *Chlamydia trachomatis* infection in women. J. Clin. Pathol. *49*:116-119.

27. **Sasavage, N.** 1994. FDA holds conference to assess HIV-1 Risks. Clin. Lab. News *11*:11-12.

28. **Schachter, J.** 1978. Medical progress: Chlamydial infections (first of three parts). N. Engl. J. Med. *298*:428-435.

29. **Schachter, J. and M. Grossman.** 1981. Chlamydial infections. Annu. Rev. Med. *32*:45-61.

30. **Stockman, L., K.A. Clark, J.M. Hunt and G.D. Roberts.** 1993. Evaluation of commercially available acridinium ester-labeled chemiluminescent DNA probes for culture identification of *Blastomyces dermatitidis, Coccidioides immitis, Cryptococcus neoformans* and *Histoplasma capsulatum*. J. Clin. Microbiol. *31*:845-850.

31. **Toye, B., R.W. Peeling, P. Jessamine, P. Claman and I. Gemmill.** 1996. Diagnosis of Chlamydia trachomatis infections in asymptomatic men and women by PCR assay. J. Clin. Microbiol. *34*:1396-1400.

32. **Vandamme, A-M., S. Van Dooren, W. Kok, P. Goubau, K. Fransen, T. Kievits, J-C. Schmit, E. De Clercq and J. Desmyter.** 1995. Detection of HIV-1 RNA in plasma and serum samples using the NASBA amplification system compared to RNA-PCR. J. Virol. Methods *52*:121-132.

33. **Walker, G.T. and M.C. Little.** 1993. Amplification systems, p. 255-297. *In* G.H. Keller and M.M. Manak (Eds.), DNA Probes, 2nd ed. Stockton Press, New York.