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Loop-Mediated Isothermal Amplification Method for Detection of Human Papillomavirus Type 6, 11, 16, and 18

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A new method was developed for detection of human papillomavirus (HPV) by loop-mediated isothermal amplification (LAMP), which was compared with the polymerase chain reaction (PCR), and real-time PCR for specificity and sensitivity. All initial validation studies with the control DNA proved to be type-specific. In order to evaluate the reliability of HPV type-specific LAMP detecting HPV DNA from clinical samples, tissue specimens were obtained from 27 patients with external genital polypoid lesions. The histologic diagnoses included condyloma acuminatum (n = 21), bowenoid papulosis (n = 2), seborrheic keratosis (n = 2), epidermolytic acanthoma (n = 1), and hairy nymphae (n = 1). HPV-6 DNA and HPV-11 DNA were detected in 18 and 3 of 21 condylomata acuminata, respectively, and there was no simultaneous infection. HPV-16 DNA was detected in one of two bowenoid papuloses. HPV DNA was not detected in the seborrheic keratoses, epidermolytic acanthoma, and hairy nymphae. These results correlated perfectly with those from real-time PCR analysis. Most positive samples contained high copy numbers of HPV DNA. HPV-11 DNA was detected in one case that could not be detected by PCR. The average reaction time was about 59 min. There was a linear correlation between the genome quantity and reaction time to reach the threshold. The LAMP method has an additional advantage as a quantitative method, and is superior in terms of sensitivity, specificity, rapidity, and simplicity, and can potentially be a valuable tool for the detection of HPV DNA. ***J. Med. Virol. 79:605–615, 2007.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: human papillomavirus (HPV); loop-mediated isothermal amplification (LAMP); polymerase chain reaction (PCR); real-time PCR

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INTRODUCTION

Human papillomavirus (HPV) is a small DNA virus which belongs to the family *Papovaviridae*, and to date, more than 100 types of HPV have been identified [Syrjanen, 2003]. Condyloma acuminatum is a benign tumor caused by low risk/mucous membranes type of HPV such as HPV-6 or 11 infecting the cutaneomucous regions of the genital and anal areas [Wang, 1993]. A diagnosis is usually made clinically, but there are many tumors that can occur in the skin and mucous membranes. Therefore, clinical diagnosis may not be sufficient, and histopathological diagnosis and virological testing are necessary. Moreover, since high-risk types of HPV associated with cervical cancer and bowenoid papulosis have been detected, identification of genotypes is preferable [zur Hausen, 2001]. Virological testing includes in situ hybridization (ISH), Southern blot hybridization method, dot blot hybridization, polymerase chain reaction (PCR), and real-time PCR [Lindh et al., 1992; Brown et al., 1993; Oliveira et al., 1994; Qu et al., 1997; Tucker et al., 2001]. However, detectability using ISH is low. In addition, PCR and real-time PCR need specific expensive equipment such as a thermal cycler, and these methods have not yet become common procedures in hospital laboratories. The loop-mediated isothermal amplification method (LAMP) is a cheap, rapid, and simple gene amplification method that was developed originally by Notomi et al. [2000] as an amplification method instead of PCR, attaining amplification efficiency and sensitivity similar to or higher than PCR. The reaction proceed between 63 and 65°C without thermocycling, and all procedures are completed in one step in about an hour when detecting the

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amplification by the appearance of turbidity in the reaction process. Recently published reports have suggested that LAMP could be an effective method for the rapid diagnosis of infectious diseases [Iwamoto et al., 2003; Kuboki et al., 2003; Maruyama et al., 2003; Parida et al., 2004]. In this study, a LAMP-based HPV type-specific DNA amplification method was developed and were compared its specificity and sensitivity with PCR and real-time PCR.

MATERIALS AND METHODS

Patients

Twenty-seven patients (19 males and 8 females; mean age, 39.1 [range, 20–80 years]) with external genital polypoid lesions entered the study at Jikei Aoto Hospital outpatient clinic between 2004 and 2005. The Ethical Review Board of the Jikei University School of Medicine approved the study, and all participants signed a written informed consent. The study was conducted according to the Declaration of the Helsinki Principles.

Tissue Processing

Biopsy specimens measuring 3 mm in diameter were taken from two lesions. One was processed for routine histopathological diagnosis. The other was stored −80°C for DNA extraction.

DNA Extraction

DNA was extracted from the specimen using QIAamp DNA Kit (Qiagen, Chatsworth, CA). After extraction, DNA was eluted in 200 μl distilled water and was stored at −20°C.

A Locations of target sequences in HPV-6, -11, -16, and -18

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HPV-6 E6   211 CACTGACCAC TGCAGAGATT TATTCATATG CATATAAACA GCTAAAGGTC CTGTTTCGAG
                        F3                                F2
           271 GCGGCTATCC ATATGCAGCC TGCGCGTGCT GCCTAGAATT TCATGGAAAA ATTAACCAAT
                                         F1
           331 ATAGACACTT TGATTATGCT GGATATGCAA CAACTGTTGA AGAAGAAACT AAACAAGACA
                        B1
           391 TTTTAGACGT GCTAATTCGG TGCTACCTGT GTCACAAACC GCTGTGTGAA GTAGAAAAGG
                        B2                B3

HPV-11 E6  101 TATGGAAAGT AAAGATGCCT CCACGTCTGC AACATCTATA GACCAGTTGT GCAAGACGTT
                        F3                      F2
           161 TAATCTTTCT TTGCACACTC TGCAAATTCA GTGCGTGTTT TGCAGGAATG CACTGACCAC
                                            F1
           221 CGCAGAGATA TATGCATATG CCTATAAGAA CCTAAAGGTT GTGTGGCGAG ACAACTTTCC
               B1                                                    B2
           281 CTTTGCAGCG TGTGCCTGTT GCTTAGAACT GCAAGGGAAA ATTAACCAAT ATAGACACTT

           341 TAATTATGCT GCATATGCAC CTACAGTAGA AGAAGAAACC AATGAAGATA T TTAAAAGT
                             B3

HPV-16 E7  601 GATTTGCAAC CAGAGACAAC TGATCTCTAC TGTTATGAGC AATTAAATGA CAGCTCAGAG
                     F3                                                F2
           661 GAGGAGGATG AAATAGATGG TCCAGCTGGA CAAGCAGAAC CGGACAGAGC CCATTACAAT
                                            F1
           721 ATTGTAACCT TTTGTTGCAA GTGTGACTCT ACGCTTCGGT TGTGCGTACA AAGCACACAC
                                  B1
           781 GTAGACATTC GTACTTTGGA AGACCTGTTA ATGGGCACAC TAGGAATTGT GTGCCCCATC
                                  B2                  B2

HPV-18 E6  371 ATTGGAAAAA CTAACTAACA CTGGGTTATA CAATTTATTA ATAAGGTGCC TGCGGTGCCA
                     F3                          F2
           431 GAAACCGTTG AATCCAGCAG AAAAACTTAG ACACCTTAAT GAAAAACGAC GATTTCACAA
                          F1                                        B1
           491 CATAGCTGGG CACTATAGAG GCCAGTGCCA TTCGTGCTGC AACCGAGCAC GACAGGAACG
                                                                       B2
           551 ACTCCAACGA CGCAGAGAAA CACAAGTATA ATATTAAGTA TGCATGGACC TAAGGCAACA
                         B3
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Fig. 1. The locations and names of the target sequences used as primers for HPV type-specific LAMP within the E6 region of HPV-6, the E6 region of HPV-11, the E7 region of HPV-16, and the E6 region of HPV-18 (**A**). Names and sequences of each primer for HPV type-specific LAMP are shown (**B**). B2c, sequence complementary to B2; F1c, sequence complementary to F1.

B Names and sequences of each primer

Name	Sequence
HPV6E6F3	5'-CACTGCAGAGATTTATTCATATGC- 3'
HPV6E6B3	5'-CGGTTTGTGACACAGGTAG - 3'
HPV6E6FIP	5'-GAAATTCTAGGCAGCACGCG-CAGCTAAAGGTCCTGTTTCG- 3'
	(B1-B2c)
HPV6E6BIP	5'-GACACTTTGATTATGCTGGATATGCCACCGAATTAGCACGTCTA- 3'
	(F1c-F2)
HPV11E6F3	5'-GTAAAGATGCCTCCACGT - 3'
HPV11E6B3	5'-CTAAGCAACAGGCACACG - 3'
HPV11E6FIP	5'-CCTGCAAAACACGCACTGAA-GACCAGTTGTGCAAGACG - 3'
	(B1-B2c)
HPV11E6BIP	5'-ACTGACCACCGCAGAGATAT-AAGGGAAAGTTGTCTCGC - 3'
	(F1c-F2)
HPV16E7F3	5'-CAGAGACAACTGATCTCTACTG - 3'
HPV16E7B3	5'-GGCACACAATTCCTAGTGT - 3'
HPV16E7FIP	5'-GTAATGGGCTCTGTCCGGTTC-AGCTCAGAGGAGGAGGAT - 3'
	(B1-B2c)
HPV16E7BIP	5'-TGCAAGTGTGACTCTACGCTT-GCCCATTAACAGGTCTTCC - 3'
	(F1c-F2)
HPV18E6F3	5'-AAAAACTAACTAACACTGGGTTA - 3'
HPV18E6B3	5'-ACTTGTGTTTCTCTGCGT - 3'
HPV18E6FIP	5'-AGGTGTCTAAGTTTTTCTGCTGG-TTTATTAATAAGGTGCCTGCG - 3'
	(B1-B2c)
HPV18E6BIP	5'-CGACGATTTCACAACATAGCTGG-GTTGGAGTCGTTCCTGTC - 3'
	(F1c-F2)

Fig. 1. (*Continued*)

Preparations of HPV DNA Templates for Control

To determine the specificity of type-specific LAMP method, 13 types of cloned HPV DNAs, HPV-1a, -2, -3, -5, -6, -10a, -11, -16, -18, -31, -33, -35, and -58, were integrated in pBR322 and were cloned by colon bacillus HB101. The plasmids were refined and extracted using QIAplep Spin Miniplep Kit (Qiagen). After extraction, DNA was eluted in 50 µl distilled water and was stored at −20°C. In addition, to determine the sensitivity of type-specific LAMP method, the DNA concentrations of HPV-6, -11, -16, and -18 were analysed on a DyNA Quant 200 fluorometer (Amesham Pharmacia Biotech, Piscataway, NJ), and the serial dilutions of each standard (HPV-6, -11, -16, and -18) were prepared to cover the range of 10^7 to 10 copies/tube.

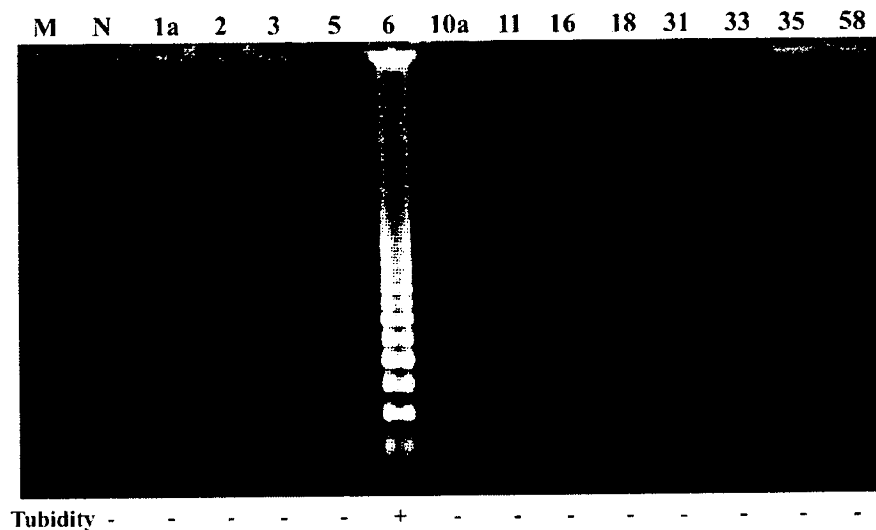
HPV Type-Specific LAMP

The LAMP reaction was conducted as described by Notomi et al. [2000] and Nagamine et al. [2002]. Specifically, the LAMP method requires a set of four primers (B3, F3, BIP, and FIP) to recognize a total of six distinct target DNA sequences (B1–B3, F1–F3) within the target DNA. LAMP primers for E6 region of HPV-6, E6 region of HPV-11, E7 region of HPV-16, and E6 region of HPV-18 were designed, using the Primer Explorer V Software (FUJITSU, Tokyo, Japan). The location and sequence of each primer in the target DNA sequences are shown in Figure 1. LAMP reactions were undertaken with a Loopamp DNA amplification kit (Eiken Chemical, Tochigi, Japan). Reaction mixtures (25 µl) contained 1.6 µM each of inner primer (FIP and BIP), 0.2 µM each of outer primer (F3 and B3), 2 × reaction mix (12.5 µl), Bst DNA polymerase (1 µl), and 5 µl of each sample. The mixtures were incubated at 63°C for 120 min. Next, turbidity was measured by TERMAMECS LA200 (Teramecs, Kyoto, Japan). The cutoff value of turbidity used to distinguish negative from positive samples was at 0.1, higher than the mean plus three SD of the turbidity of five negative samples. After turbidimetry, the LAMP products were subjected

to electrophoresis on a 1.5% SeaKem™ ME agarose (Cambrex Bio Science, Rockland, ME) gel stained with ethidium bromide. To avoid contamination between the samples, DNA extraction and LAMP were carried out in different rooms, and pipette tips with filters for aerosol prevention were used.

PCR

PCR was used for typing of HPV DNA as described by Yoshikawa et al. [1991]. The consensus primers amplifying at least nine HPV types, HPV-6, -11, -16, -18, -31, -33, -42, -52, and -58 were used for this assay, including L1C1 (5′-CGTAAACGTTTTCCCTATTTTTTT-3′), L1C2 (5′-TACCCTAAATACTCTGTATTG-3′), and L1C2M (5′-TACCCTAAATACCCTATATTG-3′). PCR was performed using 1.25U Taq DNA polymerase (TaKaRaBio-Medicals, Tokyo, Japan) by TaKaRa thermal cycler (TaKaRaBioMedicals) with 40 rounds of thermal cycling conditions; degeneration at 95°C for 1.5 min, annealing at 48°C for 1.5 min, extension at 70°C for 2 min. PCR products were confirmed by electrophoresis through 4% NuSieve™ GTG agarose (Cambrex Bio Science) gel stained with ethidium bromide. Next, they were typed on the basis of restriction fragment length polymorphisms (RFLPs) by Dde I and Rsa I.



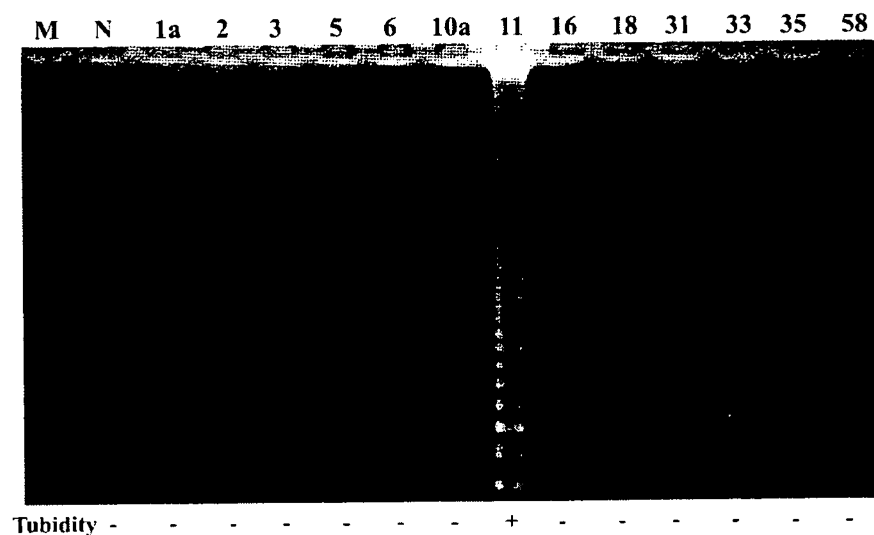
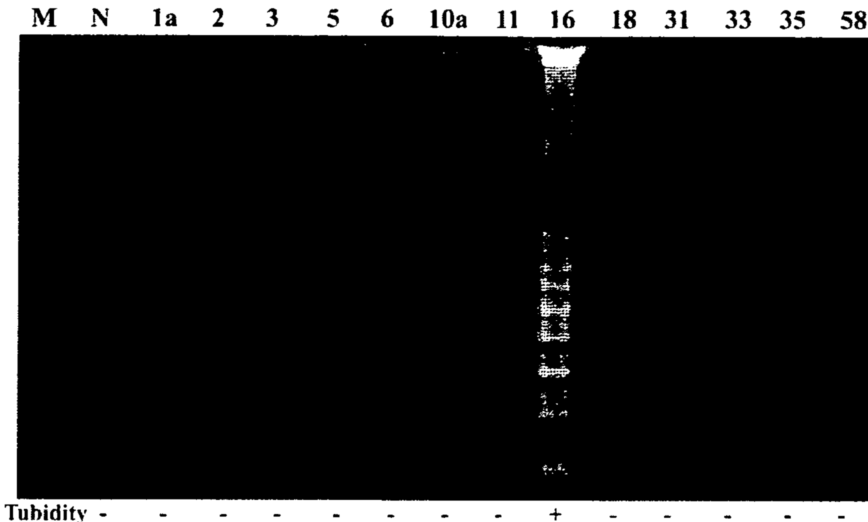


Fig. 2. DNAs extracted from HPV-1a, HPV-2, HPV-3, HPV-5, HPV-6, HPV-10a, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, and HPV-58 were amplified by using HPV-6 (**A**), HPV-11 (**B**), HPV-16 (**C**), and HPV-18 (**D**) type-specific LAMP to determine method specificity. The detection of LAMP products was assessed by agarose gel electrophoresis. The numbers indicate HPV types. M, 100-bp DNA ladder marker; N, LAMP reaction without HPV DNA.

C HPV-16 type-specific LAMP



D HPV-18 type-specific LAMP

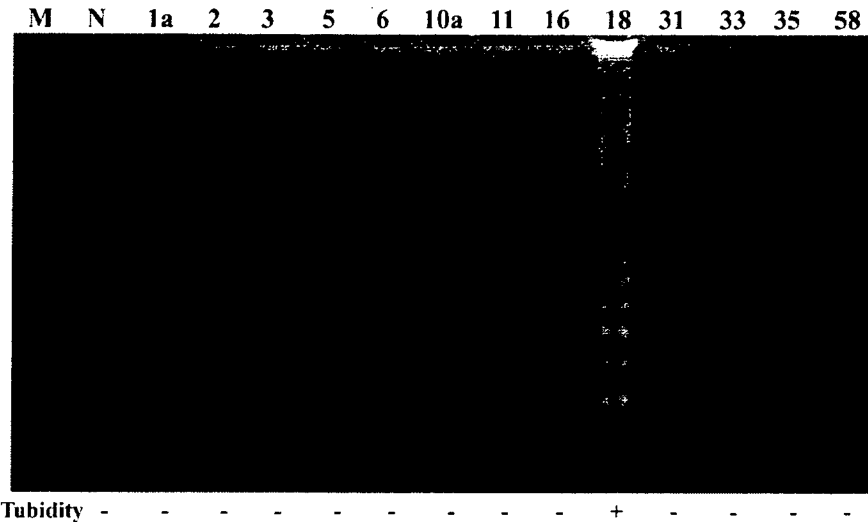


Fig. 2. (*Continued*)

Real-Time PCR for HPV-6, -11, -16, and -18

Type-specific real-time PCR was used to measure the quantity of the DNAs of HPV-6, -11, -16, and -18 in each sample. The sequences of primers and probes for E6/E7 region used have been described by Tucker et al. [2001]. PCR reactions were carried out using the TaqMan PCR Kit (PE Applied Biosystems, Foster City, CA) according to the manufacture's directions. Standard curves measuring HPV-6, -11, -16, and -18 DNA concentrations were constructed using C_T values obtained from serially diluted plasmids, pBR322, respectively, which contain the target DNA sequences. The C_T value from each sample was plotted on a standard curve, allowing automatic calculation of the copy number using Sequence Detector v1.6 software (PE Applied Biosystems). Each sample was tested in duplicate; the copy number of each sample is represented as the mean of the two values.

RESULTS

Specificity

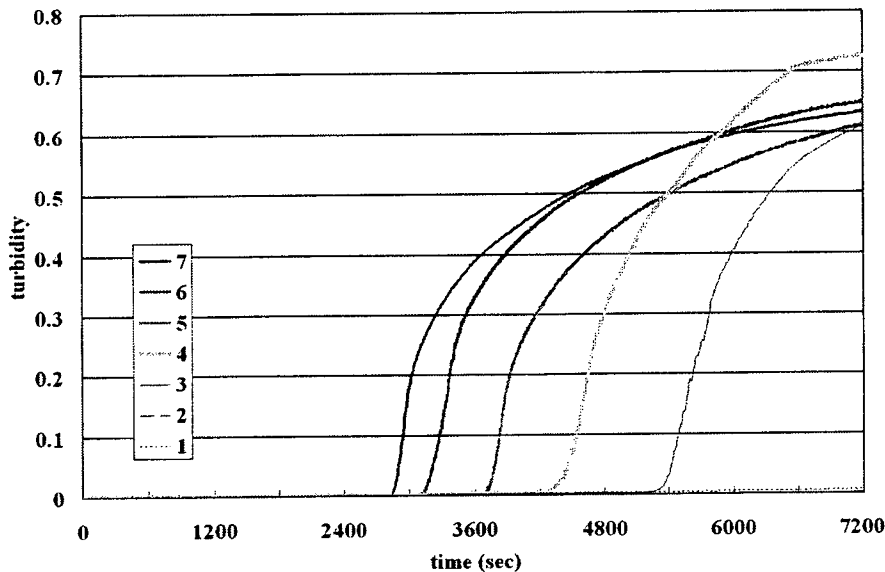
The specificity of HPV-6, -11, -16, and -18 type-specific primers was evaluated. HPV type-specific LAMP was performed by each primer on control DNA of 13 different HPV types. Each tube contained 10^7 copies of HPV DNA. As LAMP products contained several sizes of inverted-repeat structures, positive samples demonstrate a ladder pattern upon agarose gel electrophoresis. HPV type-specific LAMP primers amplified only the respective type of HPV DNA; no LAMP products were detected in reactions carried out with other type of HPV DNAs (Fig. 2).

Sensitivity

The sensitivity of PCR with consensus primer was reported to detect 0.01 pg in the previous report [Yoshikawa et al., 1991]. The sensitivity of HPV-6, -11, -16, and -18 type-specific LAMP methods were determined. Serial dilutions of the pBR322 plasmid to cover the range of 10^7 to 10 copies/tube were used to determine the detection limit of HPV type-specific LAMP. The sensitivity of HPV-6, -11, -16, and -18 type-specific LAMP determined by turbidity assay were 1,000 copies/tube (Fig. 3). In contrast, the sensitivity of HPV-6, -11, -16, and -18 type-specific LAMP determined by agarose gel electrophoresis were 100 copies/tube, 100 copies/tube, 1,000 copies/tube, and 100 copies/tube, respectively (data not shown).

Clinical Samples

Twenty-seven biopsy tissue specimens (sample numbers 1–27) collected from patients with genital polypoid lesions were examined (Table I). The histologic diagnoses included condyloma acuminatum (n = 21), bowe-



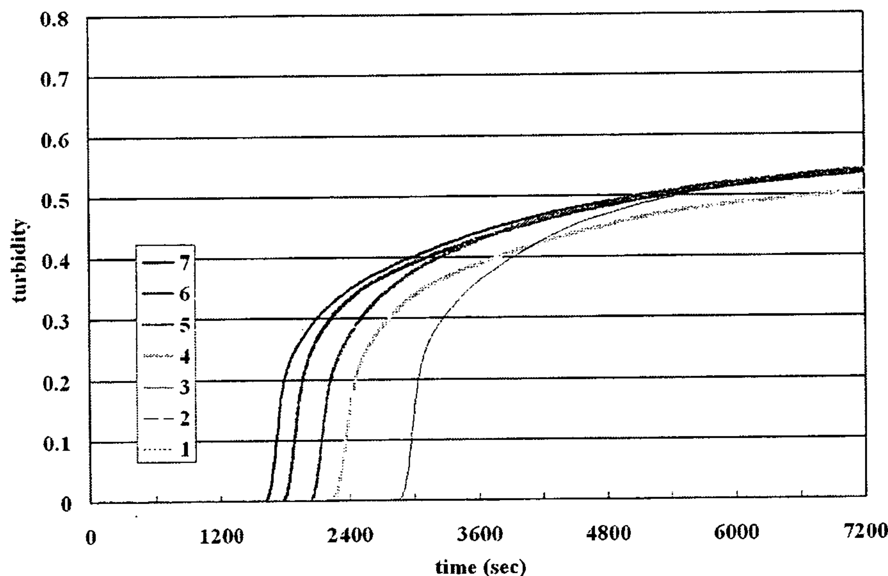
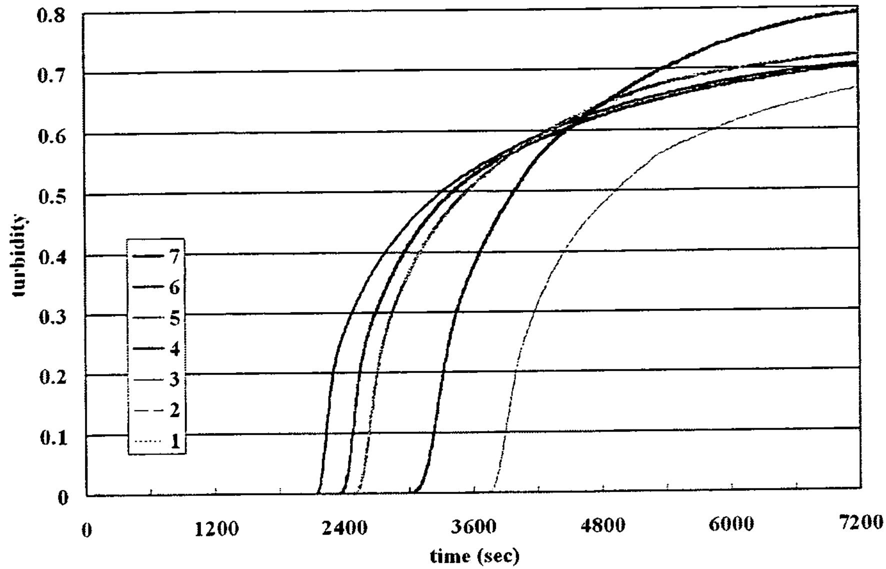


Fig. 3. To determine the sensitivities of each assay, serial dilutions of HPV-6, -11, -16, and -18 DNAs were amplified by HPV-6 (**A**), HPV-11 (**B**), HPV-16 (**C**), and HPV-18 (**D**) type-specific LAMP, respectively. The detection of LAMP products was assessed by turbidity assay using a LA-200. The numbers in the figures are the dilution of 10^n copies/tube.



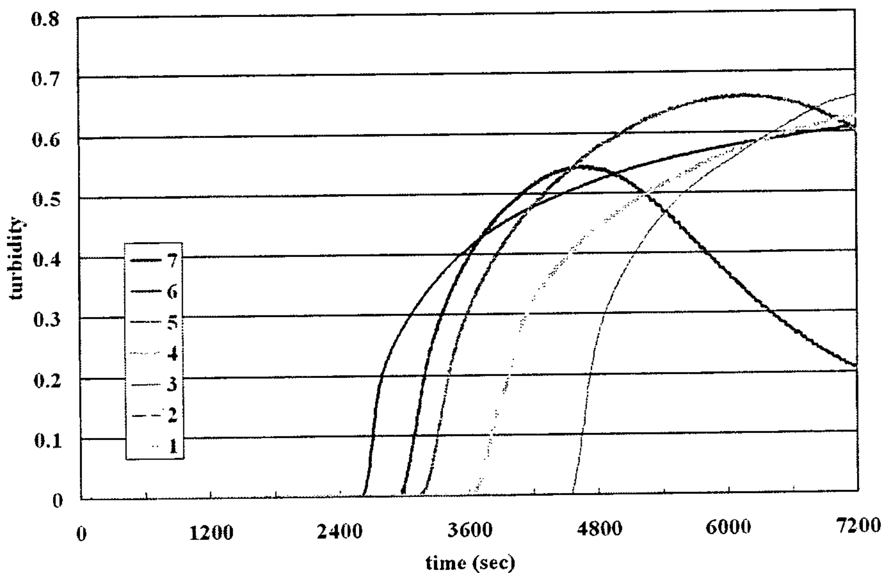


Fig. 3. (*Continued*)

noid papulosis (n = 2), seborrheic keratosis (n = 2), epidermolytic acanthoma (n = 1), and hairy nymphae (n = 1). The results of HPV type-specific LAMP were compared with those of PCR and HPV type-specific real-time PCR. The average reaction time was 59 min 9 sec. Among 21 codylomata acuminata samples, 18 (86%) were positive for HPV-6, and the remaining 3 (14%) were positive for HPV-11 by type-specific LAMP. One bowenoid papulosis specimen was positive for HPV-16 by type-specific LAMP. No HPV-18 DNA was detected in any samples. HPV type-specific LAMP amplified the respective HPV genomes and showed no cross-reactivity. HPV DNAs were detected from 22 of 27 (81%) in total, while they were detected from 21 of 27 (78%) by PCR, consisting of 18 HPV-6s, 2 HPV-11s, and 1 HPV-16 by RFLPs of PCR, identical to the results of LAMP. No HPV DNA was detected in samples that were negative by LAMP. Among the three HPV-11 positive samples by LAMP, only sample No. 21 was negative by PCR. Furthermore, the results of real-time PCR correlated perfectly with those of LAMP. Most of the positive samples by LAMP contained high copy numbers of HPV DNA. The correlation between the time (in sec) to reach the threshold >0.1 of turbidity and copy number was analyzed. The result is shown in Figure 4. There was a linear correlation between the genome quantity and reaction time to reach the threshold: y (copy numbers) = −0.001x (sec) + 9.0025.

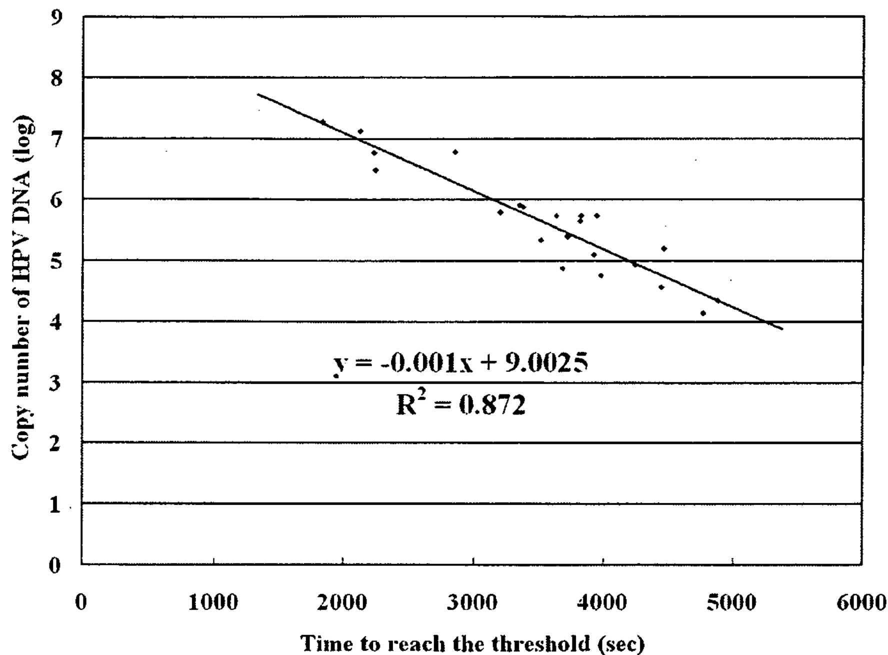


Fig. 4. LAMP reaction times were monitored to reach the threshold >0.1 in turbidity. The correlation between the logarithmic titers of copy number and reaction time is shown.

DISCUSSION

HPV is detectable in condyloma acuminatum and bowenoid papulosis, and it often becomes a problem as a sexually transmitted disease [Schwartz and Janniger, 1991; Beutner and Tyring, 1997; Beutner et al., 1998; Leung et al., 2005]. In addition, there is a high-risk type HPV that is associated with cervical cancer. Therefore, it is important to identify the genital HPV type from both an epidemiological and a public health standpoint. Virologic testings include ISH, Southern blot hybridization, dot blot hybridization, and PCR [Yoshikawa et al., 1991; Lindh et al., 1992; Brown et al., 1993; Oliveira et al., 1994]. Recently, several investigations described real-time PCR, which has the additional advantage of being a quantitative method [Cubie et al., 2001; Seth et al., 2005]. However, these methods are time-consuming, require special expensive equipment such as thermal cyclers, and may not be appropriate for testing in a clinical setting.

LAMP developed by Notomi et al. [2000] has been applied for the detection of various types of infectious agents, mainly varicella-zoster virus [Okamoto et al., 2004], SARS coronavirus [Poon et al., 2004], herpes simplex virus [Enomoto et al., 2005], measles virus [Fujino et al., 2005], mumps virus [Okafuji et al., 2005], and influenza virus [Ito et al., 2006]. LAMP proved to be rapid, highly sensitive, highly specific, and simple, suggesting that it might be used for rapid diagnosis. LAMP for the detection of HPV was developed and the sensitivity and specificity of LAMP were compared with those of PCR and real-time PCR for the detection of HPV. A pair of primers in the E1 region was designed at first, but since there is 90% homology between the PCR products of HPV-6 and HPV-11, cross-hybridization occurred between HPV-6 and HPV-11. In addition, portions of the E1 gene show greater homology and may be deleted often during HPV integration. Therefore, a pair of primers in the E6/E7 region was designed, since portions of the E6/E7 show smaller homology, and are retained and are expressed usually even when HPV integrates into cellular chromosomes. HPV type-specific LAMP amplified only the respective type of HPV DNA with no cross-reactivity. This specificity was confirmed by two independent detection methods, turbidity assay and agarose gel electrophoresis. The detection limit for HPV type-specific LAMP by the turbidity assay was 1,000 copies/tube. In contrast, the detection limit for HPV-6, -11, -16, and -18 type-specific LAMP by agarose gel electrophoresis was 100 copies/tube, 100 copies/tube, 1,000 copies/tube, and 100 copies/tube, respectively. Although the turbidity assay is less sensitive than agarose gel electrophoresis [Yoshikawa et al., 2004], the ease and rapidity of the turbidity assay make it more appropriate for clinical monitoring. Furthermore, this system also minimizes potential contamination because tubes are closed during amplification and detection of amplified DNA.

The reliability of HPV LAMP was evaluated for the detection of viral DNA from clinical samples. Since the turbidity analysis seemed to be the most appropriate test for clinical laboratory use, this assay was used for clinical sample analysis. HPV-6 DNA and HPV-11 DNA were detected in 18 and 3 of 21 condylomata acuminata, respectively, without concomitant infection. HPV-16 DNA was detected in one of two bowenoid papuloses. HPV DNA was not detected in seborrheic keratosis, epidermolytic acanthoma, and hairy nymphae. These

TABLE I. Comparison of PCR, HPV Type-Specific Real-Time PCR, and HPV Type-Specific LAMP for Detection of HPV DNA

Sample no.	Histpathological diagnosis	Typing by PCR	HPV-6			HPV-11			HPV-16			HPV-18		
			Real-time PCR (HPV-6) (copies/tube)	LAMP (HPV-6)		Real-time PCR (HPV-11) (copies/tube)	LAMP (HPV-11)		Real-time PCR (HPV-11) (copies/tube)	LAMP (HPV-16)		Real-time PCR (HPV-11) (copies/tube)	LAMP (HPV-18)	
				Turbidity (sec)	Electrophoresis		Turbidity (sec)	Electrophoresis		Turbidity (sec)	Electrophoresis		Turbidity (sec)	Electrophoresis
1	CA	HPV-6	6,180,000	2,856	+	0	—	–	0	—	–	0	—	–
2	CA	HPV-6	626,000	3,204	+	0	—	–	0	—	–	0	—	–
3	CA	HPV-6	835,000	3,348	+	0	—	–	0	—	–	0	—	–
4	CA	HPV-6	780,000	3,378	+	0	—	–	0	—	–	0	—	–
5	CA	HPV-6	221,000	3,516	+	0	—	–	0	—	–	0	—	–
6	CA	HPV-6	541,000	3,642	+	0	—	–	0	—	–	0	—	–
7	CA	HPV-6	75,900	3,690	+	0	—	–	0	—	–	0	—	–
8	CA	HPV-6	255,000	3,720	+	0	—	–	0	—	–	0	—	–
9	CA	HPV-6	453,000	3,822	+	0	—	–	0	—	–	0	—	–
10	CA	HPV-6	550,000	3,828	+	0	—	–	0	—	–	0	—	–
11	CA	HPV-6	129,000	3,924	+	0	—	–	0	—	–	0	—	–
12	CA	HPV-6	541,000	3,942	+	0	—	–	0	—	–	0	—	–
13	CA	HPV-6	57,500	3,978	+	0	—	–	0	—	–	0	—	–
14	CA	HPV-6	88,700	4,242	+	0	—	–	0	—	–	0	—	–
15	CA	HPV-6	37,900	4,446	+	0	—	–	0	—	–	0	—	–
16	CA	HPV-6	162,000	4,464	+	0	—	–	0	—	–	0	—	–
17	CA	HPV-6	14,100	4,770	+	0	—	–	0	—	–	0	—	–
18	CA	HPV-6	22,800	4,884	+	0	—	–	0	—	–	0	—	–
19	CA	HPV-11	0	—	–	18,700,000	1,830	+	0	—	–	0	—	–
20	CA	HPV-11	0	—	–	5,860,000	2,226	+	0	—	–	0	—	–
21	CA	—	0	—	–	3,140,000	2,244	+	0	—	–	0	—	–
22	BP	HPV-16	0	—	–	0	—	–	13,300,000	2,124	+	0	—	–
23	BP	—	0	—	–	0	—	–	0	—	–	0	—	–
24	SK	—	0	—	–	0	—	–	0	—	–	0	—	–
25	SK	—	0	—	–	0	—	–	0	—	–	0	—	–
26	EA	—	0	—	–	0	—	–	0	—	–	0	—	–
27	HN	—	0	—	–	0	—	–	0	—	–	0	—	–

CA, condyloma; BP, bowenoid papulosis; SK, seborrheic keratosis; EA, epidermolytic acanthoma; HN, hairy nymphae

results correlated perfectly with those from real-time PCR analysis. Most of the positive samples contained high copy numbers of viral DNA. HPV DNA was not detected in samples that were negative by LAMP. Among three HPV-11 positives by LAMP, one was negative by PCR. Therefore, the sensitivity of HPV type-specific LAMP was nearly the same as that of real-time PCR and was greater than that of PCR, for the detection of HPV infection, demonstrating the high sensitivity and specificity of HPV type-specific LAMP in the analysis of clinical samples. As for sample No. 23 of bowenoid papulosis, HPV was not detected by the LAMP method, PCR, and real-time PCR, but HPV-56 was detected by the Hybrid Capture II assay. Recently, the detection of various types of HPV has been reported [zur Hausen and de Villiers, 1994]. This LAMP method may not be useful for detection of broad HPV genotypes. However, there was a linear correlation between the genome quantity and reaction time to reach the threshold by the LAMP method, making quantitation of HPV DNA in clinical samples possible. Several studies showed that an increased HPV-16 viral load correlated with the risk for cervical carcinoma [Peitsaro et al., 2002; Ho et al., 2005]. The presence and quantity of HPV DNA are likely to be a reflection of metastasis and may have a prognostic value. Therefore, this assay may be useful to study the epidemiology, pathogenesis, and monitoring vaccine trials of HPV. The average reaction time of HPV type-specific LAMP was about 59 min, not more rapid than that of other viruses, mainly measles virus [Fujino et al., 2005], influenza virus [Ito et al., 2006], or herpes simplex virus [Sugiyama et al., 2005]. Therefore, modification of primer design or induction of loop primers may be necessary to shorten the reaction time in future analyses, as additional loop primers increase the amplification efficiency [Nagamine et al., 2001].

In summary, by setting a type-specific primer in the E6/E7 region, a new type-specific method for the detection of HPV-6, -11, -16, and -18 was developed. The sensitivity of amplification of LAMP for detection of viral DNA was nearly the same as that of real-time PCR. Cross-reactivity was not observed, and reliability of testing with clinical specimens was demonstrated. The LAMP method is superior in terms of sensitivity, specificity, speed, and simplicity, and can potentially be a valuable tool for the detection of HPV DNA in laboratories.

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REFERENCES

Beutner KR, Tyring S. 1997. Human papillomavirus and human disease. Am J Med 102:9–15.

Beutner KR, Reitano MV, Richwald GA, Wiley DJ. 1998. External genital warts: Report of the American Medical Association Consensus Conference. AMA Expert Panel on External Genital Warts. Clin Infect Dis 27:796–806.

Brown DR, Bryan JT, Cramer H, Fife KH. 1993. Analysis of human papillomavirus types in exophytic condylomata acuminata by hybrid capture and Southern blot techniques. J Clin Microbiol 31:2667–2673.

Cubie HA, Seagar AL, McGoogan E, Whitehead J, Brass A, Arends MJ, Whitley MW. 2001. Rapid real time PCR to distinguish between high risk human papillomavirus types 16 and 18. Mol Pathol 54:24–29.

Enomoto Y, Yoshikawa T, Ihira M, Akimoto S, Miyake F, Usui C, Suga S, Suzuki K, Kawana T, Nishiyama Y, Asano Y. 2005. Rapid diagnosis of herpes simplex virus infection by a loop-mediated isothermal amplification method. J Clin Microbiol 43:951–955.

Fujino M, Yoshida N, Yamaguchi S, Hosaka N, Ota Y, Notomi T, Nakayama T. 2005. A simple method for the detection of measles virus genome by loop-mediated isothermal amplification (LAMP). J Med Virol 76:406–413.

Ho CM, Yang SS, Chien TY, Huang SH, Jeng CJ, Chang SF. 2005. Detection and quantitation of human papillomavirus type 16,18 and 52 DNA in the peripheral blood of cervical cancer patients. Gynecol Oncol 99:615–621.

Ito M, Watanabe M, Nakagawa N, Ihara T, Okuno Y. 2006. Rapid detection and typing of influenza A and B by loop-mediated isothermal amplification: Comparison with immunochromatography and virus isolation. J Virol Methods.

Iwamoto T, Sonobe T, Hayashi K. 2003. Loop-mediated isothermal amplification for direct detection of Mycobacterium tuberculosis complex, M. avium, and M. intracellulare in sputum samples. J Clin Microbiol 41:2616–2622.

Kuboki N, Inoue N, Sakurai T, Di Cello F, Grab DJ, Suzuki H, Sugimoto C, Igarashi I. 2003. Loop-mediated isothermal amplification for detection of African trypanosomes. J Clin Microbiol 41:5517–5524.

Leung AK, Kellner JD, Davies HD. 2005. Genital infection with human papillomavirus in adolescents. Adv Ther 22:187–197.

Lindh E, Chua KL, Kataoka A, Bistoletti P, Groff D, Hjerpe A. 1992. Detection of human papillomavirus (HPV) using dot blot and Southern blot, hybridizing with a mixture of seven probes. Apmis 100:301–308.

Maruyama F, Kenzaka T, Yamaguchi N, Tani K, Nasu M. 2003. Detection of bacteria carrying the stx2 gene by in situ loop-mediated isothermal amplification. Appl Environ Microbiol 69:5023–5028.

Nagamine K, Watanabe K, Ohtsuka K, Hase T, Notomi T. 2001. Loop-mediated isothermal amplification reaction using a nondenatured template. Clin Chem 47:1742–1743.

Nagamine K, Hase T, Notomi T. 2002. Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol Cell Probes 16:223–229.

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28:E63.

Okafuji T, Yoshida N, Fujino M, Motegi Y, Ihara T, Ota Y, Notomi T, Nakayama T. 2005. Rapid diagnostic method for detection of mumps virus genome by loop-mediated isothermal amplification. J Clin Microbiol 43:1625–1631.

Okamoto S, Yoshikawa T, Ihira M, Suzuki K, Shimokata K, Nishiyama Y, Asano Y. 2004. Rapid detection of varicella-zoster virus infection by a loop-mediated isothermal amplification method. J Med Virol 74:677–682.

Oliveira LH, Frugulhetti IC, Passos MR, Cavalcanti SM, Fonseca ME. 1994. Human papillomavirus detection in genital lesions by in situ hybridization and ultrastructural observations. Mem Inst Oswaldo Cruz 89:195–202.

Parida M, Posadas G., Inoue S, Hasebe F, Morita K. 2004. Real-time reverse transcription loop-mediated isothermal amplification for rapid detection of West Nile virus. J Clin Microbiol 42:257–263.

Peitsaro P, Johansson B, Syrjanen S. 2002. Integrated human papillomavirus type 16 is frequently found in cervical cancer precursors as demonstrated by a novel quantitative real-time PCR technique. J Clin Microbiol 40:886–891.

Poon LL, Leung CS, Tashiro M, Chan KH, Wong BW, Yuen KY, Guan Y, Peiris JS. 2004. Rapid detection of the severe acute respiratory syndrome (SARS) coronavirus by a loop-mediated isothermal amplification assay. Clin Chem 50:1050–1052.

Qu W, Jiang G, Cruz Y, Chang CJ, Ho GY, Klein RS, Burk RD. 1997. PCR detection of human papillomavirus: Comparison between

MY09/MY11 and GP5+/GP6+ primer systems. J Clin Microbiol 35: 1304–1310.

Schwartz RA, Janniger CK. 1991. Bowenoid papulosis. J Am Acad Dermatol 24:261–264.

Seth R, Rippin J, Guo L, Jenkins D. 2005. Detection and quantitation of HPV gene expression using real-time PCR. Methods Mol Med 119:61–72.

Sugiyama H, Yoshikawa T, Ihira M, Enomoto Y, Kawana T, Asano Y. 2005. Comparison of loop-mediated isothermal amplification, real-time PCR, and virus isolation for the detection of herpes simplex virus in genital lesions. J Med Virol 75:583–587.

Syrjanen S. 2003. Human papillomavirus infections and oral tumors. Med Microbiol Immunol (Berl) 192:123–128.

Tucker RA, Unger ER, Holloway BP, Swan DC. 2001. Real-time PCR-based fluorescent assay for quantitation of human papillomavirus types 6,11,16, and 18. Mol Diagn 6:39–47.

Wang ZM. 1993. [Condyloma acuminatum: Histopathology and detection of human papilloma virus]. Zhonghua Bing Li Xue Za Zhi 22:160–162.

Yoshikawa H, Kawana T, Kitagawa K, Mizuno M, Yoshikura H, Iwamoto A. 1991. Detection and typing of multiple genital human papillomaviruses by DNA amplification with consensus primers. Jpn J Cancer Res 82:524–531.

Yoshikawa T, Ihira M, Akimoto S, Usui C, Miyake F, Suga S, Enomoto Y, Suzuki R, Nishiyama Y, Asano Y. 2004. Detection of human herpesvirus 7 DNA by loop-mediated isothermal amplification. J Clin Microbiol 42:1348–1352.

zur Hausen H. 2001. Cervical carcinoma and human papillomavirus: On the road to preventing a major human cancer. J Natl Cancer Inst 93:252–253.

zur Hausen H, de Villiers EM. 1994. Human papillomaviruses. Annu Rev Microbiol 48:427–447.

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1. 尿道炎の疫学，診断：新しい動向は？

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Ⅰ. 疫学

1. 罹患者数

男性における性感染症罹患率，特に代表的な性器クラミジア感染症（主にクラミジア性尿道炎）と淋菌感染症（主に淋菌性尿道炎）は，1993年から1995年にかけてエイズ予防キャンペーンの効果により低下したが，再び増加し，2001年から2002年には10万人あたりの年間罹患者数が160人程度となった。近年では，わずかに低下傾向がみられ，それぞれ10万人あたりの罹患者数が140人前後と推測されている。この傾向が今後も続くかどうかは明らかではないが，好ましい状況と考えられる。しかし，エイズ予防キャンペーンの効果により低下した罹患者数が，すぐに上昇に転じた経験から，今後の動向に注目する必要がある。

2. 無症候性感染

女性の性器クラミジア感染症では，無症候性感染の頻度が高いことが知られている。男性では，無症候性感染に関する報告は散見するのみであるが，2.3～7.6％の頻度とされている。われわれも，尿道炎に関する症状のない健康成人男性において，過去3ヵ月間に性交経験のあった150例を対象に調査[1]を行った。初尿を検体として核酸増幅法でクラミジア・トラコマティスと淋菌の検出を試みた。淋菌は検出されなかったが，クラミジア・トラコマティスは4.7％で検出された。この頻度は，過去の報告とほぼ同様であり，男性においても少なからず無症候性感染があることを確認した。さらに，非淋菌性尿道炎の原因微生物であるマイコプラズマ・ジェニタリウムと，非淋菌性尿道炎の原因微生物の可能性が示唆されているマイコプラズマ・ホミニス，ユレアプラズマ・ユレアリティクム，ユレアプラズマ・パルヴムについても核酸増幅法により検出を試みた[2]。マイコプラズマ・ジェニタリウムの検出は1.3％であり，過去の国内での報告と同様に低い頻度であった。マイコプラズマ・ジェニタリウムは，クラミジア・トラコマティスと比較して，尿道炎の症状を発現する頻度が高い[3]とされていることから，無症状の頻度が低かったものと考えられた。マイコプラズマ・ホミニスでは5.3％，ユレアプラズマ・ユレアリティクムでは16.0％，ユレアプラズマ・パルヴムでは29.3％と高頻度であり，真に性感染症の原因微生物であるかどうかの検討が引き続き必要になると考えられた。

3. 咽頭感染

男性の淋菌性尿道炎の感染源として，女性の咽頭淋菌が問題となってきている。淋菌性子宮頸管炎患者の咽頭拭い液を検体として培養法で調べたところ，55％で咽頭淋菌が陽性だったとの報告[4]がある。男性においても，淋菌性尿道炎患者の12％で咽頭淋菌が陽性であった[4]とされており，その意義・自然史・治療法の検討が必要となっている。

Current trend of epidemiology and diagnosis for male urethritis

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key words：尿道炎，核酸増幅法

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表1 尿道炎の症状別頻度[5]

	淋菌性尿道炎	クラミジア性尿道炎
外尿道口からの分泌物の量（比較的多い）	77.2 %	4.4 %
外尿道口からの分泌物の性状（膿性）	95.3 %	11.1 %
亀頭部の発赤あり	79.7 %	28.9 %

PCR（polymerase chain reaction）法
コバスアンプリコア STD-1
（ロシュ・ダイアグノスティックス）

TMA（transcription mediated amplification）法
アプティマ Combo2 クラミジア／ゴノレア
（富士レビオ）

SDA（strand displacement amplification）法
BD プローブテック ET　クラミジア・トラコマチス
ナイセリア・ゴノレア
（ベクトン・ディッキンソン）

図1 核酸増幅法による検出キット

II. 診断

1. 臨床症状

男子尿道炎では，特徴的な所見により，原因微生物の大まかな推測が可能であるが，原因微生物の診断が，核酸増幅法よりは感度が低い培養法や抗原検査法でされていた。そこで，われわれは，核酸増幅法での診断により，淋菌性尿道炎，（非淋菌性）クラミジア性尿道炎，非淋菌性非クラミジア性尿道炎と分類し，臨床症状を詳細に検討[5]した（**表1**）。淋菌性尿道炎では，98.4 %で外尿道口からの分泌物の量が“少量”，“中等量”，“多量”であるのに対して，クラミジア性尿道炎では95.6 %で“なし”，“少量”であった。また，淋菌性尿道炎では，95.3 %で分泌物の性状が“やや混濁”，“混濁”であったが，クラミジア性尿道炎では88.9 %で“なし”，“透明”であった。非淋菌性非クラミジア性尿道炎では，この両者のほぼ中間の症状であった。このように，臨床所見の注意深い観察によっても，ある程度の診断が可能であると考えられた。

2. 原因微生物の検出

淋菌，クラミジア・トラコマティスともに診断のための検出法は，現状では，高感度・高特異度である核酸増幅法が標準である[6]。従来は，polymerase chain reaction（PCR）法を用いた検出キットが国内では汎用されてきた。ただ，口腔内に常在する非病原性ナイセリア属を検出してしまうことから，咽頭淋菌の検出には用いることができなかった。このような状況の中で，昨年から，PCR法以外の核酸増幅法を応用した検出キットが保険適用となった（**図1**）。これらのキットは，PCR法と同様に高感度・高特異度であり，増幅阻害物質を取り除く過程を含めたり，淋菌とクラミジア・トラコマティスを同時に検出可能であるなど様々な利点を有しており，今後の応用が期待されている。また，前述した口腔内の非病原性ナイセリア属を検出することがなく，診断精度の向上が期待されている。

文献

1) Takahashi S, Takeyama K, Miyamoto S, et al: Incidence of sexually transmitted infections in asymptomatic healthy young Japanese men. J Infect Chemother **11**: 270-273, 2005
2) Takahashi S, Takeyama K, Miyamoto S, et al: Detection of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* DNAs in urine from asymptomatic healthy young Japanese men. J Infect Chemother **12**: 269-271, 2006
3) Falk L, Fredlund H and Jensen JS: Symptomatic urethritis is more prevalent in men infected with Mycoplasma genitalium than with Chlamydia trachomatis. Sex Transm Infect **80**: 289-293, 2004
4) 伊予田貴子，雑賀　威，金山明子，他：川崎市の男性および女性咽頭より分離した Neisseria gonorrhoeae の細菌学的および疫学的検討．感染症誌 **77**: 103-109, 2003
5) Takahashi S, Takeyama K, Kunishima Y, et al: Analysis of clinical manifestations of male patients with urethritis. J Infect Chemother **12**: 283-286, 2006
6) Dicker DL, Mosure DJ, Steece R, et al: Testing for sexually transmitted diseases in U. S. public health laboratories in 2004. Sex Transm Dis **34**: 41-46, 2007

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Short note

Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances

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Abstract

To simplify the molecular detection of micro-organisms, we evaluated the tolerance of loop-mediated isothermal amplification (LAMP) to a culture medium and some biological substances. The sensitivity of LAMP was less affected by the various components of the clinical samples than was polymerase chain reaction (PCR); therefore, DNA purification from samples could be omitted.
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Keywords: Loop-mediated isothermal amplification (LAMP); Biological substances; Rapid detection

In the last two decades, many antiviral compounds have been developed for the improved treatment of some viral infectious diseases; i.e., some herpes viruses, human immunodeficiency virus (HIV), influenza virus, and hepatitis B and C virus infections. At the same time, prevention strategies to protect personnel against transmission of infectious agents, especially in hospitals, have been a major focus. For success in the prevention and treatment of viral infections, the development of more rapid and accurate diagnostic methods is required. At present, polymerase chain reaction (PCR) is a common method for rapid DNA detection in clinical diagnosis. The detection of DNA in clinical samples by PCR increases the sensitivity of diagnosis of viral infectious diseases compared to that of antigenic detection or virus isolation from cell cultures. However, previous reports have reported that biological fluids and substances contained in clinical samples inhibit PCR reactions [1–4]; therefore, DNA extraction and purification from samples is required to obtain sensitive results from a PCR assay. This additional step makes it difficult to perform PCR as part of a bed-side examination, because the DNA extraction is a troublesome procedure requiring more than 30 min to complete as well as specific equipment and the added expense of a DNA extraction kit.

Recently, a new, rapid and sensitive technique named loop-mediated isothermal amplification (LAMP) has been developed [5]. DNA amplification via the LAMP reaction can be conducted under isothermal conditions ranging from 60 to 65 °C by using only one type of enzyme, the *Bst* DNA polymerase large fragment [6]. Previous reports have described LAMP assay as being able to approximately quantify the amount of target DNA [7–10], while being less affected in terms of sensitivity by the presence of inhibitory substances in clinical samples than is PCR [11,12]. To evaluate these advantages, we analyzed the tolerance of loop-mediated isothermal amplification (LAMP) against a culture medium and some biological substances for comparison with that of PCR in this study.

The evaluation was carried out using previously described LAMP and PCR reactions for herpes simplex virus type 1 (HSV-1) and a HSV-1 clinical strain, isolated from a genital lesion and propagated in an African green monkey kidney cell line (Vero cells) [4]. The virus stock was serially diluted 10-fold with Eagle's Minimum Essential Medium (MEM) and viral DNA was extracted from each diluted sample using a QIAamp DNA Mini Kit (Qiagen, Chatsworth, CA). The original virus stock contained 1.20×10^4 PFU/μl of HSV-1 and the amount of DNA extracted from the original solution was 5.36×10^6 copies/μl. The viral fluids at 10-fold dilution were used directly as samples without DNA extraction. One microliter of each sample

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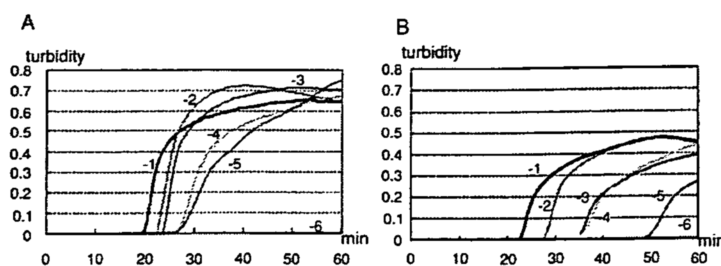

Fig. 1. Reaction profile of LAMP for detection of HSV-1 DNA from samples with (A) and without (B) DNA extraction. Viral titer and genome copy numbers of the original stocks were 1.20×10^4 PFU/μl and 5.36×10^6 copies/μl, respectively. Serial 10-fold dilutions were subjected to LAMP. The numbers in the figure represent the dilution of 10^{-n}.

was added to the reaction mixture (Loopamp DNA amplification kit, Eiken Chemical Co., Ltd., Tokyo, Japan) to a final volume of 25 μl and the reaction was performed at 65 °C for 1 h in a Loopamp real-time turbidmeter LA-200 (TERAMECS, Kyoto, Japan), as described previously [12]. The threshold for a positive spectrophotometric reading was defined as 0.1 [13].

HSV-1 DNA was detected at a 10^{-5} dilution in both samples with and without DNA extraction (Fig. 1), indicating that MEM did not affect the sensitivity of LAMP. However, the time to reach the threshold >0.1 of turbidity of the samples without DNA extraction was delayed in a dose-dependent manner when compared to those with DNA extraction. The same results were observed when saline was used to dilute the virus stock instead of MEM (data not shown). Enomoto et al. [11] reported that the sensitivity of LAMP for clinical samples without DNA extraction in MEM was much lower than that for samples with DNA extraction and concluded that swabs from clinical samples should not be placed in MEM. To clarify the discrepancy between our results and those of Enomoto et al. [11], we analyzed the effect of MEM at various concentrations in the LAMP mixture, as well as various biological substances; i.e., plasma, serum, phosphate-buffered saline (PBS), saline, urine, aqueous humor and vitreous, in comparison with PCR. The detection limit of this PCR was 100 copies/tube, as described previously [12]. Therefore, 1×10^3 copies of HSV-1 DNA were applied to each tube for both LAMP and PCR, which were 10–100 and 10-fold the detection limit, respectively. As a result, LAMP showed higher tolerance to MEM and all substances than did PCR (Table 1). Of the seven substances tested, serum, plasma and urine showed the highest level of inhibition of both LAMP and PCR, and just 1% of these completely inhibited PCR. MEM contains some components not found in PBS or saline. We speculated that some component, i.e., amino acids or salts, might inhibit LAMP and PCR reaction. In particular, PCR was more sensitive to various components than was LAMP. Therefore, when viral clinical samples are collected for molecular diagnosis and viral isolation, the swab should be placed in saline, not MEM.

Table 1
Effects of biological substances on LAMP and PCR assay

		Percentage of substances						
		0%	1%	2%	5%	10%	20%	30%
Saline	LAMP	+[a]	+	+	+	+	+	+
	PCR	+	+	+	+	+	+	−
PBS	LAMP	+	+	+	+	+	+	+
	PCR	+	+	+	+	+	−	−
MEM	LAMP	+	+	+	+	+	+	+
	PCR	+	+	−	−	−	−	−
Serum	LAMP	+	+	−	−	−	−	−
	PCR	+	−	−	−	−	−	−
Plasma	LAMP	+	+	−	−	−	−	−
	PCR	+	−	−	−	−	−	−
Urine	LAMP	+	+	−	−	−	−	−
	PCR	+	−	−	−	−	−	−
Aqueous	LAMP	+	+	+	+	+	+	+
	PCR	+	+	+	+	+	+	−
Vitreous	LAMP	+	+	+	+	+	+	−
	PCR	+	+	+	−	−	−	−

[a] +, positive; −, negative.

Since the development of PCR, molecular diagnosis has become a standard clinical test. Moreover, many applications and modifications of PCR have been reported, including quantitative real-time PCR. In this study, we estimated the merits of LAMP, compared with those of PCR, and found that the tolerance of LAMP for biological substances was superior. This suggests that the DNA extraction step can be omitted in LAMP assay. To date, this troublesome and expensive procedure has prevented the spread of some valuable techniques for the detection the pathogens in small-scale hospital laboratories and private clinics. The omission of the DNA extraction step would save both time, labor and cost and would allow LAMP assay to be developed as an auxiliary diagnostic method for the bed-side as well as for outpatients.

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References

[1] Mercier B, Gaucher C, Feugeas O, Mazurier C. Direct PCR from whole blood, without DNA extraction. Nucleic Acids Res 1990;18:5908.
[2] Khan G, Kangro HO, Coates PJ, Heath RB. Inhibitory effects of urine on the polymerase chain reaction for cytomegalovirus DNA. J Clin Pathol 1991;44:360–5.

[3] Ruano G, Pagliaro EM, Schwartz TR, Lamy K, Messina D, Gaensslen RE, et al. Heat-soaked PCR: an efficient method for DNA amplification with applications to forensic analysis. Biotechniques 1992;13:266–74.
[4] Wiedbrauk DL, Werner JC, Drevon AM. Inhibition of PCR by aqueous and vitreous fluids. J Clin Microbiol 1995;33:2643–6.
[5] Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 2000;28:E63.
[6] Nagamine K, Watanabe K, Ohtsuka K, Hase T, Notomi T. Loop-mediated isothermal amplification reaction using a nondenatured template. Clin Chem 2001;47:1742–3.
[7] Hong TC, Mai Q, Cuong DV, Parida M, Minekawa H, Notomi T, et al. Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. J Clin Microbiol 2004;42:1956–61.
[8] Mori Y, Kitao K, Tomita N, Notomi T. Real-time PCR turbidimetry of LAMP reaction for quantifying template DNA. J Biochem Biophys Methods 2004;59:145–57.
[9] Fujino M, Yoshida N, Yamaguchi S, Hosaka N, Ota Y, Notomi T, et al. A simple method for the detection of measles virus genome by loop-mediated isothermal amplification (LAMP). J Med Virol 2005;76:406–13.
[10] Okafuji T, Yoshida N, Fujino M, Motegi Y, Ihara T, Ota Y, et al. Rapid diagnostic method for detection of mumps virus genome by loop-mediated isothermal amplification. J Clin Microbiol 2005;43:1625–31.
[11] Enomoto Y, Yoshikawa T, Ihira M, Akimoto S, Miyake F, Usui C, et al. Rapid diagnosis of herpes simplex virus infection by a loop-mediated isothermal amplification method. J Clin Microbiol 2005;43:951–5.
[12] Kaneko H, Iida T, Aoki K, Ohno S, Suzutani T. Sensitive and rapid detection of herpes simplex virus and varicella-zoster virus DNA by loop-mediated isothermal amplification. J Clin Microbiol 2005;43:3290–6.
[13] Mori Y, Nagamine K, Tomita N, Notomi T. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. Biochem Biophys Res Commun 2001;289:150–4.

特集　各領域の最新診療ガイドライン

性感染症

松田　静治*

はじめに

近年，HIV 感染をはじめ性感染症（STD）の世界的増加が大きな社会的関心を招いているが，この背景には性の自由化，性風俗の変化，性行為の多様化といった風潮が根底にある．STD の抱える問題点として，病原微生物の多様化，無症状感染の広がりや性器外感染（咽頭感染など）の増加に加えて，患者の低年齢化，つまり性行動の活発な若年層での流行が懸念されている．

1．性感染症の現況

近年，若年層の間で STD の急速な増加が問題になっている[1~3]．STD には 10 種以上の疾患があり，その内訳は梅毒をはじめ細菌性疾患では淋菌感染症，性器クラミジア感染症が，ウイルスによるものでは性器ヘルペス，尖圭コンジローマ，エイズ（HIV），肝炎（HBV）などがあり，加えて原虫による腟トリコモナス症，真菌による性器カンジダ症，寄生虫による毛ジラミがある．さらに産婦人科で近年注目されている細菌性腟症も広義には性関連疾患として STD に含まれる．

このように STD の病原微生物は多様化し，細菌ではクラミジア・トラコマチス，淋菌が，ウイルスではヘルペスウイルス群，パピローマウイルスなどが主流である．疾患別に増えている疾患は女性の性器クラミジア感染症と男性の淋菌感染症などがあり[1~3]，なかでも最近は女性患者の増加が注目される（図 1）．一方，梅毒は近年激減している[1]．また，HIV（エイズ）は日本で 1985 年に初めて報告があって以来着実に増え続け（図 2），ほかの STD と同じく，HIV においても若年層での女性の割合が高いことが注目される[1]．

2．ガイドラインを踏まえた治療と留意事項

日本性感染症学会では，1996 年に CDC のガイドライン（現在 2006 年版が発表されている）[5]を参考に性感染症の検査，治療方針を作成したが，保険適用における日本の慣行の投与法との不整合などがあり，その後再作成を行い，2004 年，2006 年[4]には薬剤耐性淋菌の増加，新しい検査法と薬剤の登場を踏まえ，さらに改定を行った．

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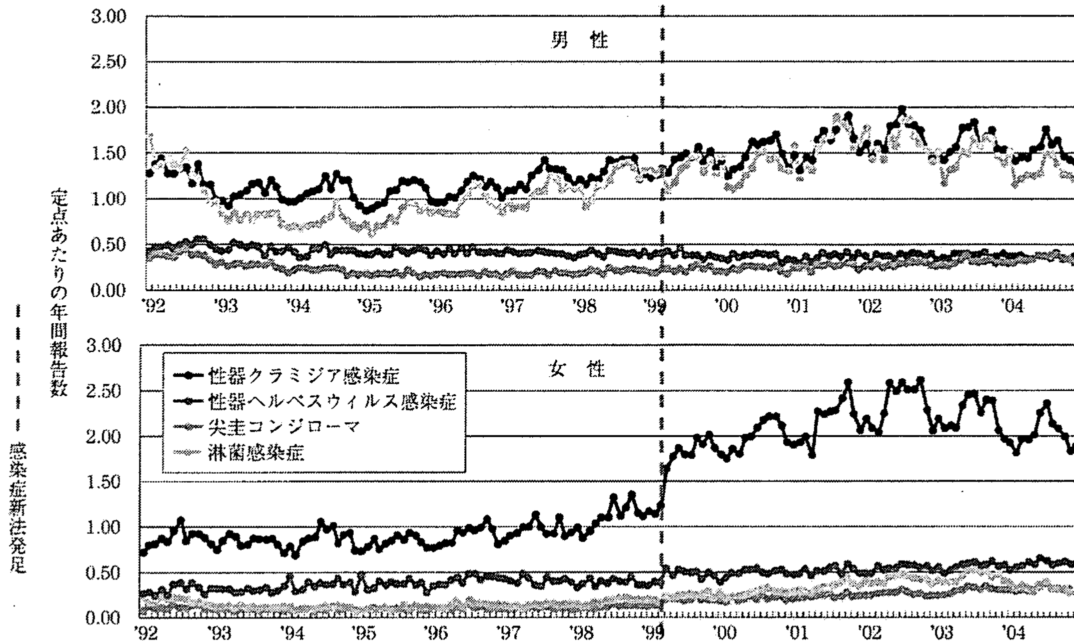

図1 性感染症の年次推移
(感染症発生動向調査 2004年12月末まで)

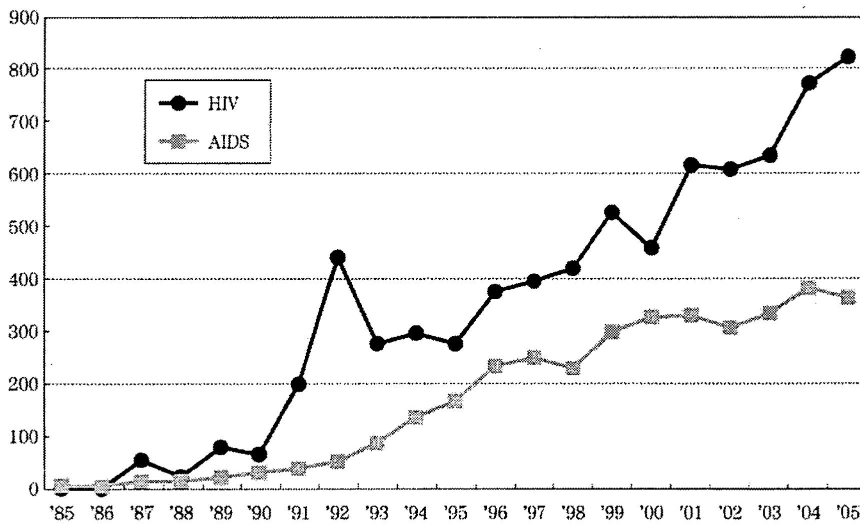

図2 新規HIV感染者およびエイズ患者数の年次推移
(2005年エイズ動向委員会報告)

以下に疾患別のガイドラインを述べる．

1 性器クラミジア感染症

1）疫学，症状，診断

性器クラミジア感染症は最も頻度の高いSTDで，子宮頸管炎は男性の尿道炎とともに感染力と汚染度の高いSTDと考えられている．感染後1～3週間で発症し，時に上行感染し，子宮付属器炎など骨盤内炎症性疾患（PID），肝周囲炎を起こし，卵管性不妊や子宮外妊娠の誘因になったりする．また妊婦の子宮頸管炎から時に母子感染

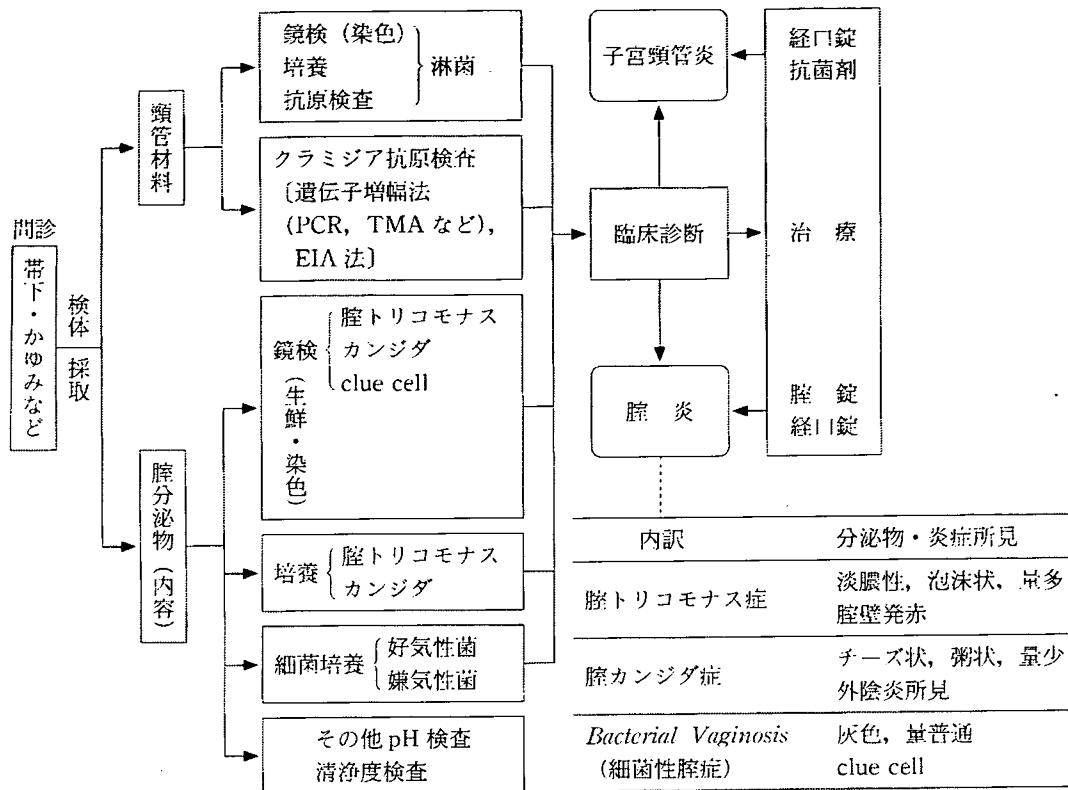

図 3 子宮頸管炎，腟炎の診断手順

（産道感染による児の結膜炎，肺炎）を起こしたりするが，近年抗原陽性妊婦に対する抗菌薬投与で母子感染は減少した．男性ではクラミジアによる尿道炎は非淋菌性尿道炎の約半数以上を占め，淋菌性尿道炎におけるクラミジアの合併頻度は20～30％と言われる．性器クラミジア感染症では，主訴（帯下感など）が少なく，2/3 が無症状であり，早期検診，検査が蔓延を食い止める最善の策である．図 3 の子宮頸管炎の診断手順のように，診断は通常子宮頸管分泌物について，抗原検査法（TMA 法[6]，PCR 法などの核酸増幅法や核酸検出法，EIA 法など）を用いて行う．問題は子宮頸管炎と思われる症例にすでに silent PID をその時点で合併，続発している場合で，抗原検査だけでの PID の診断や経過観察には注意が必要である（抗体検査の併施必要）．

2）治 療

治療には経口投与が原則で，マクロライド系のアジスロマイシン（AZM）（1,000 mg 単回投与のみ），クラリスロマイシン（CAM）（1 日 400 mg/分 2，7 日間）やニューキノロン系のガチフロキサシン（GFLX）（1 日 400 mg/分 2，7 日間），レボフロキサシン（LVFX）（1 日 300 mg/分 3，7 日間）を使用する[7]．ただ，妊婦には上記のマクロライド系を使用する（**表 1**）．治癒判定として投与開始 2 週間後の抗原検査でクラミジアの陰性化を確認する．血清抗体検査では治癒判定はできない．またパートナーの検査，治療も同時に進めることが望ましい．なおテトラサイクリン薬は尿道炎と異なり，子宮頸管炎，子宮付属器炎の適応がないが，骨盤腹膜炎の場合にミノサイクリンの点滴が使用できる．

2 淋菌感染症

1）疫学，症状，診断

淋菌（*Neisseria gonorrhoeae*）による感染症は STD の代表的疾患の一つで，通常潜伏期間は数

表1　性感染症の治療法（その1）

	起炎菌	治療薬	使用法	備考
性器クラミジア感染症	クラミジア・トラコマチス	（経口）		近年ペニシリン，ニューキノロン，セフェム耐性の淋菌が増加している．クラミジア・トラコマチスには今のところ耐性株はない．
		アジスロマイシン（AZM）	1回1,000 mg 単回	
		クラリスロマイシン（CAM）	1回200 mg　1日2回（7日間）	
		※ガチフロキサシン（GFLX）	1回200 mg　1日2回（7日間）	
		※レボフロキサシン（LVFX）	1回100 mg　1日3回（7日間）	
		※トスフロキサシン（TFLX）	1回150 mg　1日2回（7日間）	
淋菌感染症	淋菌	（注射）		
		セフトリアキソン（CTRX）	1回1,000 mg 静注（単回）	
		セフォジジム（CDZM）	1回1,000 mg 静注（単回）	
		スペクチノマイシン（SPCM）	1回2,000 mg 筋注（単回）	

※妊婦には使用しない．

（性感染症診断・治療ガイドライン2006参照）

日～7日である．女性では子宮頸管炎，男性では尿道炎が代表である．淋菌は時に管内性に感染を拡大し，骨盤内炎症性疾患（PID）や精巣上体炎，さらに咽頭炎，直腸炎を起こしたりする．患者数では男性が多いが，最近は女性の淋菌感染症が増加していることに注意すべきである．

症状は，典型的な子宮頸管炎では帯下感の主訴と頸管帯下として粘液性，膿性の分泌物が外子宮口付近に見られるが，患者の多くは感染の自覚がない．PIDはクラミジアやほかの一般細菌に比べて淋菌の起炎菌としての比率は低いが，症状，病態（発熱，下腹痛，付属器圧痛など）は，クラミジアによるものより強い．

検査法には子宮頸管分泌物の培養や抗原検査法（TMA法[6]，PCR法などの核酸増幅法，核酸検出法）があるが（**図3参照**），グラム染色標本の鏡検での診断は男性の尿道炎に比べて正診率は低い．咽頭炎を疑えば咽頭材料の培養を行う．この際，抗原検出法は非病原ナイセリアの存在で，偽陽性を起こすこともあり，不適当である．

2）治　療

近年淋菌の抗菌薬耐性化は著しく，多剤耐性化も進み[8]，使用薬剤は限られるようになった．すなわちニューキノロン薬では70％に達する耐性が見られ，テトラサイクリン系や多くのセフェム薬でも耐性が増加し，ペニシリン系もプラスミド性ペニシリン耐性（PPNG），染色体性ペニシリン耐性（non-PPNG）とも近年耐性が増加し，もはやペニシリン製剤は本症に対する第一選択薬ではない．現在勧められる治療法は注射薬の単回投与が主流で，セフトリアキソン（CTRX）1,000 mg静注単回投与，セフォジジム（CDZM）1,000 mg静注単回投与，スペクチノマイシン（SPCM）2,000 mg筋注単回投与がある（**表1**）．いずれも半減期が長く，単回投与が可能である．PIDに対しては，単回にこだわらず投与期間は症例ごとに判断すべきで，特にほかの細菌との複数菌感染が予想される場合，クラミジアにも有効な薬剤（例えばニューキノロン薬）の併用も考慮する．