（表７）流行抑制のための集団免疫率

感染症	潜伏期間（日）	基本再生産数（R_0）	集団免疫率(%)
麻疹	7～16	16～21	90～95
ムンプス	8～32	11～14	85～90
風疹	7～28	7～9	80～85
ポリオ	2～45	5～7	80～86
天然痘	9～45	5～7	80～85
百日咳	5～35	16～21	90～95
ジフテリア	2～30	6～7	85
インフルエンザ	1～10	2～3	67*
水痘	10～21	8～10	90？

集団免疫率＝$(1-1/R_0)\times 100$

*小学生の集団

る人，消極的に接種する人，積極的に拒否する人（宗教的理由または思想信条による）の三群にわかれる．積極的に接種する人は約30%，積極的に拒否する人は約１%であり，接種率を高めるためには，消極的に接種する人にアプローチすることが大切である．

まとめ

現場でワクチンを接種する際に問題となるのは，ホストの免疫状態と接種するワクチンが生ワクチンか不活化ワクチンかである．臨床の現場でよく遭遇する事例をもとに，ワクチン接種の考え方をレビューした．

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Vaccination to the individuals in special clinical circumstances

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Abstract

Excess limitation could be adapted on vaccination, since several clinicians might be afraid of adversed events after vaccination. The factors, which clinicians should consider on vaccination, are the immunological status of vaccinees and the type of vaccine, such as live vaccine or inactivated vaccine. Infants and children, pregnant women, the elderly, and individuals with chronic diseases are immune-altered and could be administered with live and inactivated vaccines, except pregnant women, who could not be administered with live vaccine. Only the immunocompromised individuals are contraindicated with live vaccines. Since influenza vaccine produced in Japan is well purified, anaphylaxis could not be induced in children with egg-allergy theoretically. In inactivated vaccines, priming with two or three doses via three to eight weeks interval is important for induction of specific immunity. The best way that protects immunocompromised individuals, who could not be administered with live vaccines, is elimination of vaccine preventable diseases by high vaccination rate, which overwhelms the herd-immunity.

Key words : immune-altered, immunocompromised, egg allergy, pregnancy, prime and boost

感染症・予防接種レター（第52号）

日本小児保健協会予防接種・感染症委員会では「感染症・予防接種」に関するレターを毎号の小児保健研究に掲載し，わかりやすい情報を会員にお伝えいたしたいと存じます。ご参考になれば幸いです。

日本小児保健協会予防接種・感染症委員会

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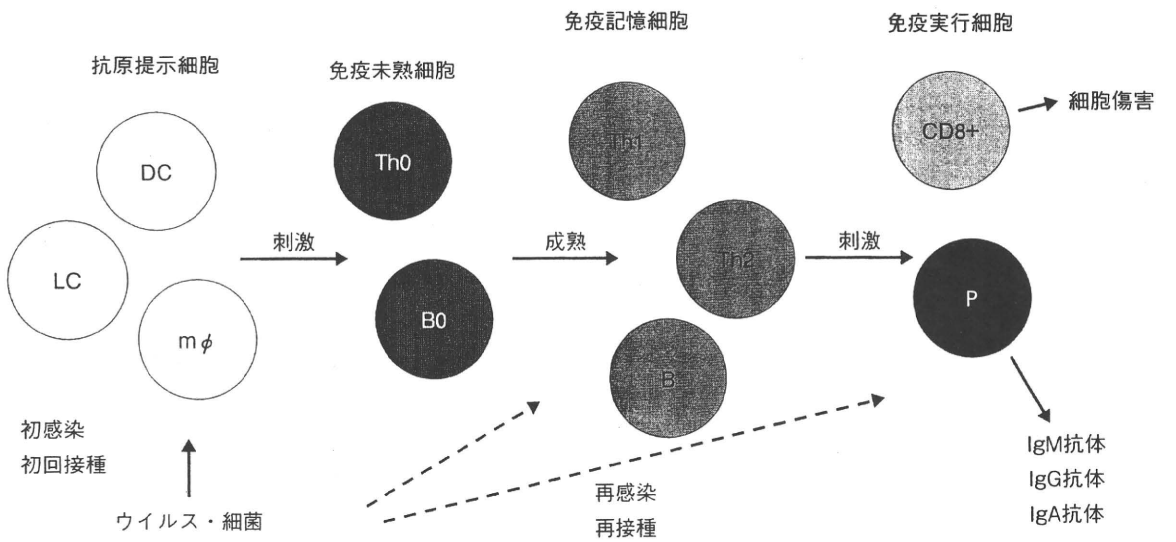
ワクチンと免疫

はじめに

適切な抗原で作成されたワクチンを接種すると，まず各種サイトカインの誘導を含めた自然免疫が働き，同時に特異的細胞性免疫や抗体などの獲得免疫が誘導される。スケジュールから外れたとき，基礎疾患を有する者にワクチンを接種するときなどは，免疫の知識が必要になる。本稿ではワクチンと免疫について解説する。

1．免疫プライミングとブースティング

免疫に関係する細胞群には，抗原提示細胞，免疫未熟細胞，免疫記憶細胞，免疫実行細胞がある。抗原提示細胞は，接種されたワクチン抗原を認識すると同時に免疫未熟細胞に刺激を与え，免疫未熟細胞を免疫記憶細胞に成熟させる働きがある（図1）[1,2]。成熟した免疫記憶細胞は免疫実行細胞を刺激し，抗体を産生させ，特異的細胞性免疫を誘導する。免疫未熟細胞を成熟させ免疫記憶細胞を誘導し，免疫実行細胞により抗体を産生させる段階が免疫プライミングであり，誘導された免疫記憶細胞を刺激し，免疫実行細胞の数を増加させ，抗体を更に高める段階がブースティングである。免疫実行細胞は刺激がないと数は減少するが，一度誘導された免疫記憶細胞は喪失しないため，DPT ワクチ



DC：樹状細胞，LC：ランゲルハンス細胞，mφ：マクロファージ，P：プラズマ細胞，Th0：未熟ヘルパーT細胞，Th1：1型ヘルパーT細胞，Th2：2型ヘルパーT細胞，B0：未熟B細胞，B：B細胞，CD8+：CD8+T細胞
1）一度免疫記憶細胞が誘導されると，抗体価が陰転化しても1回の接種で二次免疫応答が起こる
2）記憶B細胞の誘導には4～6ヵ月が必要なため，追加接種（ブースティング）は初回接種後4～6ヵ月以降に行う

図1　感染・ワクチンと特異免疫の誘導

ン，日本脳炎ワクチンなどの不活化ワクチンで，1期初回終了後の追加接種を忘れたときは，数年後でも気がついたときに1回追加接種をすれば免疫ブーステイングが誘導される。スケジュールから外れたとき，不活化ワクチンでは必要な接種回数を接種することが基本である。

2．生ワクチンとキラーT細胞（$CD8^+$細胞）の誘導

感染からの回復に$CD8^+$細胞が必要な感染症である麻疹，水痘，ムンプス，風疹などでは，$CD8^+$細胞が誘導できる生ワクチンの接種が必須である。生ワクチンを接種すると，MHCクラスⅠが関係する抗原提示細胞が働き$CD8^+$細胞が誘導される。なお，不活化ワクチンや結合型ポリサッカライドワクチンでは特異的細胞性免疫は誘導できるが，誘導できるのはMHCクラスⅡが関係する$CD4^+$細胞だけである。

3．抗体レベルとブースター

抗体を持っている人に生ワクチンを接種すると，接種を受けた人の抗体価に応じて抗体のブースターがかかる人とかからない人がいる。生ワクチンを接種して抗体のブースターがかかるとは，接種したワクチン株が増殖した結果である。低い抗体価だと，接種されたウイルスが増殖して抗体のブースターが誘導され，高い抗体価では接種したウイルスが増殖しないため抗体のブースターが誘導されない。麻疹と風疹では，発症予防レベルやブースターがかからないレベル（感染予防レベル）は示されているが（表1）[3]，水痘やムンプスでは発症予防レベルや感染予防レベルは未確定である。なお，年齢が低いほど感染予防に必要な抗体価が高く（高い抗体価でもブースターがかかる），成人は小児よりも低い抗体レベルで感染が予防される[4]。

不活化ワクチンでも抗体価が低いと抗体のブースターがかかるが，抗体価が高いとブースターがかかりにくい。また，インフルエンザ2009（H1N1）pdmウイルスワクチンの臨床研究結果から，不活化ワクチンでは一度ブースターが誘導されると，3週間後に追加接種しても抗体の更なる上昇は認められない[1]。免疫寛容が働くためと考えられている。

表1　麻疹・風疹の発症予防レベル・感染予防レベル

測定方法	抗体価		
	陽性	発症予防	感染予防
麻疹			
文献（IU/ml）		≧120～200	≧500～1,000
NT（倍）	2	≧4	≧32
PA（倍）	16	≧64	≧512
EIA（EIA価）	2.0	≧4.0	≧16.0
HI（倍）	8	≧8	≧16
風疹			
文献（IU/ml）	4	≧4～10	≧15～25
LA（IU/ml）	4	≧10	≧15～25
HI（倍）	8	≧16	≧32
EIA（EIA価）	2.0	≧5.0	≧7.5～12.5

4．代表的なワクチン予防可能疾患の発症予防レベル

感染防御には，抗体で表わされる液性免疫だけではなく，細胞性免疫や粘膜免疫も関与している。しかし，細胞性免疫や粘膜免疫の測定は手間がかかるため，測定が容易な抗体で感染防御力を示している（表2）[5]。表2で示す抗体価は，多くの人の発症を予防する抗体価であり，曝露されたウイルス量が多いときは，発症予防には高い抗体価が必要である。また，全身感染症では，感染を受けると同時に免疫の二次応答も始まるため，相対的に低い抗体価で発症を予防できるが，局所性感染症では，感染による二次免疫応答が始まるまでに症状が出現するため，発症予防のためには比較的高い抗体価が必要である。インフルエンザでHI抗体価40倍は50％の人の，160倍は90％以上の人の発症を予防する抗体価である[6]。また，麻疹抗体120mIU/mlや風疹抗体4～15IU/mlは95％以上の人の発症予防レベルである。発症者と密接に接触する機会が多い医療従事者は，曝露されるウイルス量が多い危険性があり，発症予防のために表1で示す抗体価よりも高い抗体価が必要である。

5．ワクチンとLow Responder（低反応者）

ワクチンを接種しても一部の人では発症予防レベルの抗体価が誘導できないことがある。このような人はLow Responder（低反応者）と呼ばれ，遺伝的因子が関係している。接種時の抗体価が表2に示した発症予防レベル以下で

表2 代表的なワクチン予防可能疾患の発症予防レベル

ワクチン	抗体測定方法	必要な抗体価
ジフテリア	中 和	0.01～0.1IU/ml
A型肝炎	EIA	10mIU/ml
B型肝炎	EIA	10mIU/ml
Hib結合型	EIA	0.15μg/ml
インフルエンザ	HI	40倍
日本脳炎	中 和	10倍
麻 疹	マイクロ中和	120mIU/ml
ムンプス		未確定
百日咳	EIA (PT)	5単位
肺炎球菌	EIA, opsonophagocytosis	0.20～0.35μg/ml (小児), 8倍
ポリオ	中 和	4～8倍
狂犬病	中 和	0.5IU/ml
ロタウイルス		未確定
風 疹	免疫沈降	10～15IU/ml
破傷風	中 和	0.1IU/ml
水 痘	FAMA, gp ELISA	64倍, 5IU/ml*
黄 熱	中 和	5倍

Plotkin SA : Clin Vaccine Immunol 2010 ; 17 : 1055-1065.
*参考値

あっても，麻疹ワクチンや風疹ワクチンを追加接種したとき抗体価が発症予防レベル以上に賦活されない場合がある。低い抗体価でも他の免疫機能が働き，接種されたウイルスが体内で増殖しなかったためと考えられ，理論上抗体価が発症予防レベル以下でも発症しない人である。

不活化ワクチンであるHBワクチンでもLow Responderは認められる。3回接種しても抗体が陽性にならない（HBs抗体＜10mIU/ml）場合は，プライミングしたワクチンと異なる遺伝子型で製造されたワクチンで4回目を接種すると抗体価が上昇することがある。4回接種しても抗体価が上昇しない人は，HBウイルスが感染しにくい人である。

ま と め

ワクチンと免疫について解説した。不活化ワクチンではプライミングとブースティングが大切である。また，発症予防は測定が容易な抗体で評価されているが，細胞性免疫や粘膜免疫も関与しており，抗体価が低くても発症が予防される人は存在する。

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Short communication

Development of a novel TaqMan real-time PCR assay for detecting rubella virus RNA

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ABSTRACT

Although the number of cases of rubella and congenital rubella syndrome has decreased recently in Japan, both are still important health problems. To control rubella infection, a rapid and reliable method for diagnosis of rubella is required as soon as possible. Direct detection of the viral genome in clinical samples is viewed as crucial for laboratory diagnosis. In this study, a novel diagnostic method for rubella virus, based on a fluorogenic real-time PCR (TaqMan) assay, was developed, and its sensitivity for various virus strains was compared with that of a conventional RT-PCR. The new assay allowed more rapid and sensitive detection of the virus than did the conventional RT-PCR, and could detect at least 10 pfu of the native strains in Japan (1a, 1D, 1j).

Rubella is caused by infection with *Rubella virus* (RV), and the symptoms include a mild rash and a fever. The most severe effect of RV infection is the congenital rubella syndrome (CRS), which is caused by infection during early pregnancy and may result in abortion, miscarriage, stillbirth, and severe birth defects. The most common congenital defects are sensorineural deafness, heart disease, and cataracts. RV belongs to the genus *Rubivirus*, family *Togaviridae*, and possesses a positive-sense, single-stranded RNA with a 9.8-kb nucleotide length that contains two open reading frames (ORFs). The 5′- and 3′-ORFs encode the nonstructural proteins p150 and p90 (Liang and Gillam, 2000; Pugachev et al., 1997) and the structural protein capsid, E1 and E2, respectively (Frey, 1994; Oker-Blom et al., 1984; Yao et al., 1998).

In Japan, single-dose rubella vaccination was started in 1976, and two-dose vaccination against measles and rubella by a combined Measles and Rubella (MR) vaccine was introduced in 2006. In 2008, a national vaccination campaign to control measles and rubella was introduced, with the aim of eliminating measles and decreasing rubella by the year 2012. To support this effectively, an active surveillance system including a rapid and reliable method for laboratory confirmation is essential. Current laboratory diagnosis employs mainly serological methods that measure anti-rubella IgM, although it takes at least 5 days after the onset of infection to detect a specific increase in anti-rubella IgM. Detection of viral RNA, for example, in oral fluid, is possible by RT-PCR if samples are obtained during the first 4–5 days after the onset of a rash (CDC, 2008). Rubella symptoms are similar to those of other viral infections (e.g. measles virus, echovirus, or parvovirus B19), making it difficult to differentiate rubella from other viral infections on the basis of clinical symptoms alone (WHO, 2007a). Other viruses such as measles and parvovirus B19 sometimes give rise to false-positive IgM results (Tipples et al., 2004).

Therefore, detection of the RV genome is considered to be a promising approach for monitoring rubella because of its sensitivity and specificity. Although several RT-PCR assays for the detection of the RV genome in clinical specimens have been described (Cooray et al., 2006; Jin and Thomas, 2007; Vyse and Jin, 2002; Zhu et al., 2007), the conventional methods require a long time and multiple procedures, and are susceptible to carry-over contamination when multiple samples, including positive samples, are tested. Real-time PCR does not require post-PCR sample handling and seems to be more feasible for much faster and higher-throughput assays. Although some investigators have described real-time PCR methods for detecting the RV genome (Abernathy et al., 2009; Hübschen et al., 2008; Rajasundari et al., 2008; Zhao et al., 2006), there has been no report confirming the sensitivity and specificity between various viral strains, or excluding the possibility of false-positive results for other viruses exhibiting similar symptoms. In this study, a novel real-time PCR system that included one-step RT-PCR for the diagnosis of rubella infection was developed and the sensitivity was compared with that of a conventional nested RT-PCR, which covers the sequencing region for genotyping, using synthesized RNA and various virus strains. This system permits detection of a minimum of 10 copies of a positive control RNA and 1 pfu of RV.

Twenty-six complete sequences of RV accessible on GenBank were aligned, and a highly conserved region was selected in design-

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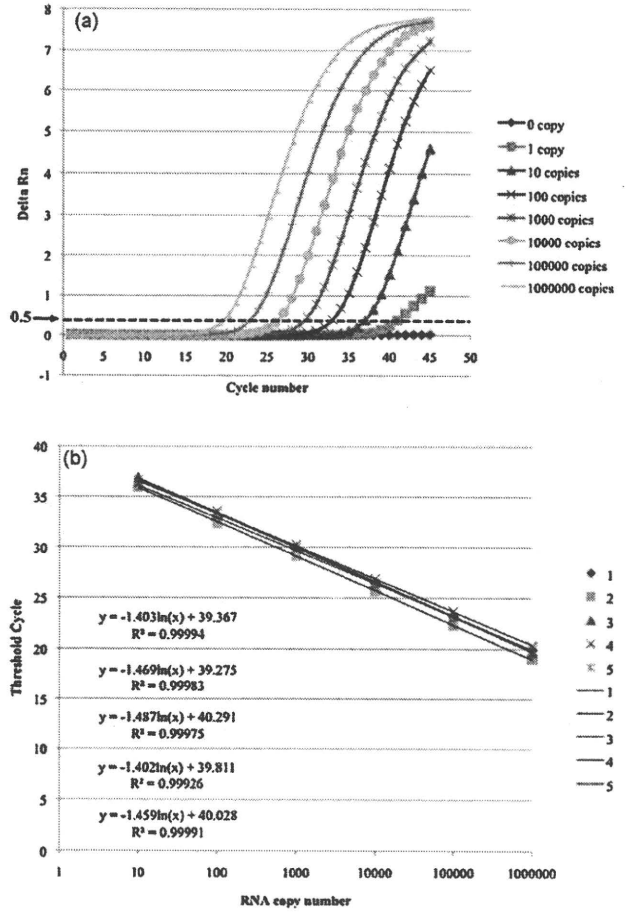

Fig. 1. Determination of the cut-off values (a) and standard curves (b). Ten-fold dilutions of standard RNA were used with the TaqMan assay. (a) Each sample was amplified in triplicate. The broken line indicates $\Delta R_n = 0.5$. (b) Five independent experiments were performed in triplicate. Each numerical expression represents a regression line, and R^2 indicates the coefficient of determination.

ing TaqMan primers and probes using Primer Express Software Ver. 3 (Applied Biosystems, Foster City, CA). The sequence of the probe was 5′-CCGTCGGCAGTTGG-3′ (encoding nt 93–106), that of the forward primer was 5′-CCTAHYCCCATGGAGAAACTCCT-3′ (nt 32–54), and that of the reverse primer was 5′-AACATCGCGCACTTCCCA-3′ (nt 143–160). The reporter probe was conjugated with 6-carboxyfluorescein and minor groove binder at the 5′ and 3′ termini, respectively.

To determine the cut-off values for the TaqMan assay, synthesized RNA in vitro (consisting of 1–782 nt of TO336 wt-type) was quantified by OD_{260}, and a 10-fold dilution series was prepared (from 2.0×10^5 to 2.0×10^{-1} copies/μl) and applied to the TaqMan assay (Fig. 1a). The assay was performed by using a TaqMan RNA-to-C_T 1-Step Kit (Applied Biosystems) in a total volume of 20 μl, which contained a final concentration of 900 nM sense and antisense primers, 250 nM probe, 1× TaqMan RT Enzyme Mix, and 1× TaqMan RT-PCR Mix. The kinetics of cDNA amplification were monitored using an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems) under the conditions of 48 °C for 15 min, 95 °C for 10 min, and 45 cycles of 95 °C for 15 s and 60 °C for 1 min. This assay was carried out in triplicate for each sample, including a no-template control. Test samples were considered positive if amplification with a threshold cycle (Ct) value <40 and ΔR_n signal >0.5 were seen in all of the triplicate reactions. Five distinct sets of 10-fold-diluted standard RNA samples (1.0×10^6 to 1 copies/reaction) were examined to estimate the dynamic range of this assay. Although above 10 copies/reaction were detected in all samples, 1 copy/reaction was detected in 47% of samples (Table 1). The standard curve from 1 to 1.0×10^6 copies/reaction did not satisfy a linear relationship of $R^2 > 0.99$ (data not shown); therefore, the data of one copy/reaction was omitted from the standard curve. Log-linear regression plots showed a strong linear relationship ($R^2 > 0.99$) between the log of the starting copy number (from 10 to 1.0×10^6 copies) and the Ct values (Fig. 1b). All standard curves showed a similar slope and intercept, and the reproducibility of the standard curve was reliable, at least in the range from 10 to 1.0×10^6 copies/reaction. These results indicate that the dynamic range of this assay was from 10 to at least 1.0×10^6 copies/reaction.

Thirteen RV genotypes have been recognized to date (WHO, 2007b), all belonging to only one serotype. These genotypes are classified into two clades: clade1 and clade 2. Clade 2 has not been reported in Japan, except for Rvi/OSAKA.JPN/11.07 (2B), isolated in 2007. Few studies using real-time PCR for detection of the RV genome have compared the sensitivity and specificity between the viral genotypes or strains using RNA extracted from viral stocks, although several investigators have used real-time PCR for RV diagnosis (Abernathy et al., 2009; Hübschen et al., 2008; Rajasundari et al., 2008; Zhao et al., 2006). To investigate the sensitivity and specificity of the TaqMan assay for various viruses, 10 RV strains consisting of five genotypes (1a, 1B, 2B, 1D and 1j), which included vaccine strains (Shishido and Ohtawara, 1976) that have been isolated, propagated, and titrated in our laboratory, were examined using this assay, and the results were compared with a conventional RT-PCR. The viral RNA was prepared from 140 μl of culture medium of RK-13 cells infected with each RV, using a QIAamp Viral RNA Mini Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's protocol, extracted with 70 μl of elution buffer, and each 5 μl of eluted sample was subjected to the TaqMan assay and the conventional RT-PCR. The conventional RT-PCR conditions are described below.

For amplifying an 851-nt region in the E1 coding region (nt 8702–9553) using a primer set F1 (5′-CGACGCGGCCTGCTGGGGC) and R9 (5′-AGGTCTGCCGGGTCTCCGAC), RT-PCR was performed with One-Step RT-PCR Kit (QIAGEN) according to the manufacturer's protocol, except that the total reaction volume and concentration of the primers were altered to 25 μl and 0.3 μM, respectively. After carrying out the RT reaction for 30 min at 55 °C and denaturation for 15 min at 95 °C, the reaction mix-

Table 1
Detection limit of the TaqMan assay using a series of synthesized standard RNA.

Standard RNA (copies/reaction)	Number of positive samples/number of tested samples					Positive (%)
	1	2	3	4	5	
10^6	3/3	3/3	3/3	3/3	3/3	100%
10^5	3/3	3/3	3/3	3/3	3/3	100%
10^4	3/3	3/3	3/3	3/3	3/3	100%
10^3	3/3	3/3	3/3	3/3	3/3	100%
10^2	3/3	3/3	3/3	3/3	3/3	100%
10	3/3	3/3	3/3	3/3	3/3	100%
1	0/3	2/3	0/3	3/3	2/3	47%

tures were incubated for 35 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 1 min, followed by 72 °C for 10 min. The RT-PCR product (0.5 μl) was used as a template for a nested PCR with 2 mM $MgCl_2$, 50 mM KCl, 25 mM TAPS buffer (pH 9.3), 200 μM sense (5′-CAGCACCCTCACAAGACCGTC-3′) and anti-sense (5′-CACAGCAGTGGTGTGTGTGCC-3′) primers, and 0.025 U Ex Taq polymerase (TaKaRa Bio, Shiga, Japan). The cycling conditions were as follows: 98 °C for 30 s, and 30 cycles of 98 °C for 10 s and 68 °C for 1 min. The PCR products were resolved on a 1% agarose gel and visualized by ethidium bromide staining. As a result, at least 1 pfu of RV could be detected in Rvi/OSAKA.JPN/11.07 (2B), SRL6.97 (1D), and Osaka' 94 (1D) strains by the TaqMan assay (Table 2), although a greater viral load was required in the other viral strains (from 10 to 100 pfu). On the other hand, at least 10 pfu of the virus was required for detection by the conventional RT-PCR, and for the Kagoshima' 04 strain (1j), as much as 1000 pfu of the virus was required. Thus, the novel real-time PCR had about 10-fold greater sensitivity compared with the conventional RT-PCR. Although the conventional RT-PCR amplified a relatively long region (851 bp), the sensitivity of this method was almost identical to that of another RT-PCR amplifying 481 bp (data not shown). Conventional RT-PCR amplifying the 851-nt sequencing region in the E1 coding region could be used for direct genotyping. Thus, the conventional RT-PCR is also thought to be useful for rapid genotyping. The sequences of the target region for the TaqMan assay were identical among genotype 1a strains (data not shown). The reason for the low sensitivity of the TaqMan assay for the TO336 wild-type and the Matsuura vaccine strains is unknown.

It is necessary for monitoring to confirm the morbidity of rubella precisely, and it is especially important to distinguish rubella from other infectious diseases. Clinical diagnosis of rubella is sometimes confused with measles, HHV-6, and parvovirus B19, because they have similar major symptoms to rubella (WHO, 2007a). Therefore, to exclude the possibility of false-positive results for other viruses, measles viral RNA (strains SA203, Yamagata, IC-B, YS-4, Edmonston, and Toyoshima, kindly provided by Dr. Seki, Department of Virology III, National Institute of Infectious Diseases), HHV-6 type A DNA (kindly provided by Dr. Inoue, Department of Virology I, National Institute of Infectious Diseases), and parvovirus B19 DNA (kindly provided by Dr. Okada, Department of Blood, National Institute of Infectious Diseases) were examined (Table 3). Measles virus strains used in this study included wild-type isolates with various genotypes that are found frequently in Japan or Asia (D5, D9, and H1) (CDC, 2005), a vaccine strain (A), and a laboratory strain (D3). Although the amount of template in the measles virus and parvovirus B19 was not known precisely, the samples were confirmed as positive by conventional RT-PCR or PCR-specific for each virus (data not shown). These viral RNAs or DNAs were not detected by the probe or primers used in this study.

It is difficult to obtain an adequate number of clinical samples from patients suspected of rubella infection in Japan, because the morbidity rate decreased significantly in recent years. Therefore, to mimic the detection of viral RNA extracted from clinical specimens, a spike test was performed as an alternative resource. Throat swabs from healthy donors collected in a Universal Viral Transport (UVT) medium (BD, Franklin Lakes, NJ) were added to 1, 10, 10^2, and 10^3 pfu of three viral strains, TO336 vaccine (1a), Osaka' 94 (1D), and Kagoshima' 04 (1j), and subjected to the TaqMan assay and the conventional RT-PCR. Ten pfu of all viruses could be detected by the TaqMan assay (Table 4). However, even 10^3 pfu of each virus could not be detected by the conventional RT-PCR. The TaqMan assay detected viral RNA from the spiked test samples to almost the same degree as with a viral culture medium. This showed that neither the RNA extraction step nor the TaqMan assay was affected by the presence of contaminants included in clinical specimens (i.e., throat swabs).

Table 2
Sensitivity of the TaqMan and conventional RT-PCR assay.

Strain	Genotype	Viral dose (pfu)											
		10^4		10^3		10^2		10^1		10^0		10^{-1}		10^{-2}	
		TaqMan	RT-PCR	TaqMan	RT-PCR	TaqMan	RT-PCR	TaqMan	RT-PCR	TaqMan	RT-PCR	TaqMan	RT-PCR	TaqMan	RT-PCR
TO336 wild	1a	27.5 ± 0.25	N/A	31.3 ± 0.30	+	35.4 ± 0.25	+	40.0<	−	40.0<	−	40.0<	N/A	40.0<	N/A
TO336 vaccine	1a	24.2 ± 0.26	N/A	28.2 ± 0.17	+	31.8 ± 0.26	+	35.4 ± 0.15	−	40.0<	−	40.0<	N/A	40.0<	N/A
Matsuura wild	1a	26.6 ± 0.34	N/A	30.3 ± 0.52	+	34.1 ± 0.51	+	37.5 ± 0.50	+	40.0<	−	40.0<	N/A	40.0<	N/A
Matsuura vaccine	1a	28.1 ± 0.48	N/A	31.6 ± 0.39	+	35.5 ± 0.53	+	40.0<	−	40.0<	−	40.0<	N/A	40.0<	N/A
SRL 8.79	1B	25.0 ± 0.94	N/A	28.6 ± 0.86	+	32.4 ± 0.79	+	36.0 ± 1.00	+	40.0<	−	40.0<	N/A	40.0<	N/A
Rvi/OSAKA.JPN/11.07	2B	22.1 ± 0.42	N/A	25.2 ± 0.29	+	28.9 ± 0.21	+	32.7 ± 0.39	+	36.2 ± 0.15	−	40.0<	N/A	40.0<	N/A
SRL 6.97	1D	24.4 ± 0.34	N/A	27.5 ± 0.24	+	31.0 ± 0.19	+	35.0 ± 0.08	−	38.8 ± 0.15	−	40.0<	N/A	40.0<	N/A
Osaka' 94	1D	23.7 ± 0.31	N/A	27.3 ± 0.22	+	31.0 ± 0.21	+	34.6 ± 0.35	+	38.5 ± 1.11	−	40.0<	N/A	40.0<	N/A
Miyazaki' 01	1j	25.3 ± 0.16	N/A	29.0 ± 0.19	+	32.9 ± 0.21	+	36.5 ± 0.22	+	40.0<	−	40.0<	N/A	40.0<	N/A
Kagoshima' 04	1j	26.3 ± 0.18	N/A	29.8 ± 0.19	+	33.5 ± 0.10	−	37.3 ± 0.41	−	40.0<	−	40.0<	N/A	40.0<	N/A

Table 3
Specificity of the TaqMan assay.

Virus			Amount of template	Ct	Result
RV					
	TO336 wild	1a	100 pfu	35.4 ± 0.25	Pos.
	TO336 vaccine	1a	100 pfu	31.8 ± 0.26	Pos.
	Matsuura wild	1a	100 pfu	34.1 ± 0.51	Pos.
	Matsuura vaccine	1a	100 pfu	35.5 ± 0.53	Pos.
	SRL 8.79	1B	100 pfu	32.4 ± 0.79	Pos.
	Rvi/OSAKA.JPN/11.07	2B	100 pfu	28.9 ± 0.21	Pos.
	SRL 6.97	1D	100 pfu	31.0 ± 0.19	Pos.
	Osaka' 94	1D	100 pfu	31.0 ± 0.21	Pos.
	Miyazaki' 01	1j	100 pfu	32.9 ± 0.21	Pos.
	Kagoshima' 04	1j	100 pfu	33.5 ± 0.10	Pos.
MV					
	SA203	D5	N/A	40.0<	Neg.
	Yamagata	D9	N/A	40.0<	Neg.
	IC-B	D3	N/A	40.0<	Neg.
	YS-4	H1	N/A	40.0<	Neg.
	Ed	A	N/A	40.0<	Neg.
	Toyoshima	A	N/A	40.0<	Neg.
Others					
	HHV-6 U1102	A	0.5 μg of DNA	40.0<	Neg.
	HHV-6 Z29	B	0.5 μg of DNA	40.0<	Neg.
	Parvovirus B19		N/A	40.0<	Neg.

Table 4
Spike test for detection of RV in throat swabs.

Strain	Genotype	Viral dose (pfu)			
		10^3		10^2	
		TaqMan	RT-PCR	TaqMan	RT-PCR
TO336 vaccine	1a	29.4 ± 0.13	–	33.1 ± 0.11	–
Osaka' 94	1D	27.8 ± 1.12	–	32.1 ± 1.33	–
Kagoshima' 04	1j	28.5 ± 1.12	–	32.7 ± 1.45	–

Table 5
Detection of RV in clinical specimens by TaqMan assay.

Individuals	Specimens	TaqMan #1	Ct	TaqMan #2	Ct	RT-PCR	Virus isolation
1	Oral fluid	Pos.	34.9	Pos.	35.7	Pos.	Pos.
2	Oral fluid	Pos.	39.4	Pos.	39.3	Neg.	Pos.
3	Oral fluid	Pos.	38.4	Pos.	37.9	Neg.	Pos.
4	Oral fluid	Pos.	36.1	Pos.	36.7	Pos.	Pos.
5	Oral fluid	Pos.	38.5	Pos.	39.1	Neg.	Pos.
6	Oral fluid	Pos.	31.6	Pos.	33.7	Neg.	Pos.

To compare the sensitivity of the novel TaqMan assay in clinical specimens with the conventional RT-PCR, six oral fluids collected from an outbreak of rubella in 2004 in Japan were tested using the TaqMan assay (TaqMan #1) and conventional RT-PCR. All samples were confirmed as positive by the conventional RT-PCR after passages in culture. As a result, all specimens (6/6) were positive using the TaqMan assay, although two of the six samples were positive using the conventional RT-PCR (Table 5). The same samples were also subjected to another TaqMan assay (TaqMan #2), which has been reported previously (Zhu et al., 2007; Abernathy et al., 2009). However, no amplification was detected both in the negative control and in the positive control using the TaqMan #2 method (data not shown). When the primers and probe were used with the same reagent in this study (TaqMan RNA-to-C_T 1-Step Kit), amplification signals were obtained. Under these conditions, all specimens (6/6) were also found to be positive, with similar Ct values to the TaqMan #1 method (Table 5). Although it was unclear why the primers and probe of TaqMan #2 method did not function under the assay conditions described previously, the primers and probe could function equally well as those of the novel TaqMan method (TaqMan #1) under the assay condition used in TaqMan #1 method. The TaqMan assay used in this study was more reliable, because it was confirmed that this assay could detect various viral strains sensitively and did not cross-react with other viral RNAs causing similar symptoms as rubella.

Detection of viral RNA in oral fluid seems to be more suitable for rapid diagnosis of rubella. WHO does not recommend viral genome detections for laboratory confirmation, probably due to the possibility of laboratory contamination and cross-contamination when performing RT-PCR followed by nested PCR. However, given the lower risk of contamination using real-time PCR and its higher sensitivity compared with conventional RT-PCR, the former technique is concluded to be more suitable for rapid diagnosis. The new real-time PCR assay described above was able to detect at least 10 copies of RV RNA and 1 pfu of virus. This TaqMan PCR assay is considered to be useful for rapid diagnosis and screening of rubella when used in conjunction with conventional RT-PCR.

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