JOURNAL OF CLINICAL MICROBIOLOGY, July 2004, p. 3017–3022 Vol. 42, No. 7
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.7.3017–3022.2004

Measles Virus Genotyping by Nucleotide-Specific Multiplex PCR

Jacques R. Kremer,[1,2] Fred Fack,[1] Christophe M. Olinger,[1] Mick N. Mulders,[1]† and Claude P. Muller[1,2]*

Department of Immunology and WHO Collaborative Centre for Measles, Laboratoire National de Santé, L-1011 Luxembourg, Luxembourg,[1] and Ecole doctorale BioSE, Université Henri Poincaré, F-54505 Vandoeuvre–lès-Nancy Cedex, France[2]

Received 3 February 2004/Returned for modification 26 February 2004/Accepted 4 April 2004

A simple genotyping method based on multiplex PCR has been developed to discriminate between all active measles virus (MV) clades and genotypes (A, B3.1, B3.2, C2, D2-D9, G2-G3, and H1-H2). The sequencing reaction was replaced by six multiplex PCRs: one to identify the clade and five to identify the respective genotype. Primers were sensitive to clade- and genotype-specific nucleotides and generated fragments of type-specific sizes that were analyzed by conventional agarose gel electrophoresis. On the basis of all published MV sequences, positive and negative predictive values of 99.2% and 98.6% were calculated. Variability in the primer binding sites, which could potentially reduce sensitivity, was very limited among published sequences. As new genotypes are described, additional specific primers can be included in the multiplex PCR with relative ease. Although sequencing remains the "gold standard," the present method should facilitate MV genotyping especially in developing countries and will therefore contribute to enhanced MV control and elimination strategies as recommended by the World Health Organization.

Global measles incidence has dramatically decreased after the introduction of routine vaccination, but endemic circulation persists in many developing countries in Asia and Africa (10, 12). To reduce measles mortality, the World Health Organization (WHO) has renewed its effort for global measles control on the basis of enhanced vaccination and surveillance. Molecular epidemiology of measles virus (MV) has proven very useful for monitoring the efficiency of vaccination campaigns, for monitoring routes of transmission, and for proving local interruption of virus circulation (13).

MV is a monotypic morbillivirus belonging to the family of *Paramyxoviridae*. The negative-stranded nonsegmented RNA genome encodes eight proteins, including the nucleocapsid (N) and the hemagglutinin (H) proteins. Sequence diversity within the complete H gene and the 450 C-terminal nucleotides of the N gene (nucleotides [nt] 1233 to 1682) (11) classifies MV strains into eight clades (A to H) containing 22 different genotypes (A, B1 to B3, C1 and C2, D1 to D9, E, F, G1 to G3, and H1 and H2). Most MV genotypes have a more or less characteristic geographic distribution. Genotypes B1, B2, D1, E, F, and G1 are considered to be inactive (25).

MV sequence information from many (developing) countries with a persistently high measles incidence is limited. A simple genotyping method, without the need for sequencing, could potentially contribute to a more complete picture of MV genotype distribution throughout the world and thus enhance MV control programs. The WHO recommends that the genotype of representative strains should be determined from every outbreak (25).

Few alternative methods, limited to one or a few genotypes, have been described for MV. Samuel et al. developed a method based on a modification of the amplification refractory mutation system to genotype D6 strains (20). Takahashi et al. used restriction fragment length polymorphism to distinguish between genotypes C1, D3, and D5 (22). Here we propose a genotyping strategy based on clade- and genotype-specific multiplex PCR to discriminate between all currently circulating MV genotypes.

MATERIALS AND METHODS

RNA isolation and RT-PCR. Viruses were obtained from the American Type Culture Collection, Manassas, Va. (U01987, clade A [18]); A. D. M. E. Osterhaus, Rotterdam, The Netherlands (AF193513, genotype D4 [24]; AF171232, G2 [24]; AF193512, H1 [24]); A. Tischer, Berlin, Germany (AF474930, D7 [21]); T. Whistler, Johannesburg, South Africa (U64582, D2 [9]); D. Chibo, Victoria, Australia (AF481485, D9 [5]); and P. A. Rota, Atlanta, Ga. (M89921, C2 [1]; U01977, D3 [18]; L46758, D5 [17]; L46750, D6 [17]; AY184217, G3 [4]; AF045217, H2 [26]). The remaining strains were isolated in our laboratory by standard cocultivation on B95a cells (AJ232203, B3.1; AJ232209, B3.2 [7]; AJ250070, D8 [24]) as described previously (7). RNA was extracted from 200 μl of virus culture supernatant (RNeasy Kit; Qiagen, Leiden, The Netherlands) according to the manufacturer's protocol. Specific cDNA of MV nucleoprotein mRNA was synthesized by reverse transcription (RT) with Moloney murine leukemia virus reverse transcriptase (Invitrogen, Merelbeke, Belgium) and primer MVN8 (TTA TAA CAA TGA TGG AGG, nt 1740 to 1722). The cDNA was further amplified by using the primers MVN8 and NP3seq (TTG CTG GTG AGT TAT CCA CAC TTG, nt 973 to 996) to obtain a 768-bp fragment. This was followed by a nested PCR with MVN1 (GAT GGT AAG GAG GTC AGC TGG, nt 1208 to 1228) and MVN7 (TCG GCC TCT CGC ACC TA, nt 1699 to 1683), respectively, to increase sensitivity of detection and specificity.

Clade- and genotype-specific PCR. The product of the MVN1/MVN7-nested PCR of the virus of interest was included as a template (2 μl, 1:100 diluted) into 15 μl of a PCR mixture containing 1.7 mM $MgCl_2$, 1× PCR buffer (Invitrogen), 0.5 mM deoxynucleoside triphosphate, 0.6 U of Platinum *Taq* DNA polymerase (Invitrogen) to perform the cladetyping and genotyping multiplex PCRs (Fig. 1). The same common antisense primer revCG (0.4 μM) (GGGTGTCCGTGTCT GAGCCTTG, nt 1650 to 1629) was used in all reactions. Different combinations

* Corresponding author. Mailing address: Department of Immunology and WHO Collaborative Centre for Measles, Laboratoire National de Santé, 20A, rue Auguste Lumière, L-1011 Luxembourg, Luxembourg. Phone: 352-490604. Fax: 352-490686. E-mail: claude.muller@lns.etat.lu.

† Present address: WHO European Regional Office, Copenhagen, Denmark.

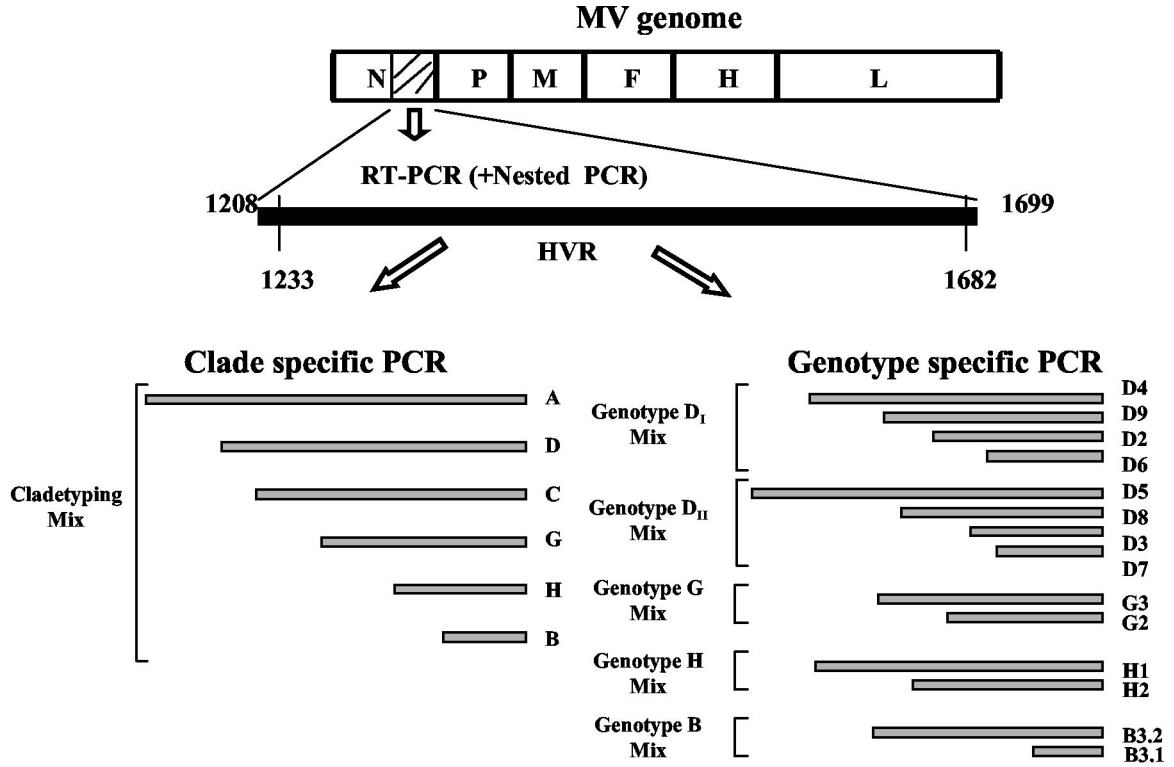

FIG. 1. Strategy for clade- and genotyping of MV by multiplex PCR with type-specific primers. Bars represent relative fragment lengths. N, nucleoprotein; P, phosphoprotein; M, matrix protein; F, fusion protein; H, hemagglutinin protein; L, large protein.

of sense primers were included into either the cladetyping or the different genotyping multiplex PCR mixtures at the concentrations given in Table 1. After an initial denaturation step at 95°C for 2 min, amplification was performed by using 25 cycles of sequential denaturation (30 s at 95°C), annealing (10 s at 66°C), and elongation (10 s at 72°C). The reaction was carried out on a Mastercycler Gradient (Eppendorf, Hamburg, Germany). PCR fragments were separated in a 3.5% agarose gel (Invitrogen). The fragment size was determined in comparison to the 1-Kb Plus DNA Ladder (Invitrogen).

RESULTS

Clade- and genotype-specific nucleotides. Clade- and genotyping was based on type-specific single nucleotide differences between clades or genotypes of MV. All published sequences of the hypervariable region (HVR) of the MV N gene (nt 1233 to 1682) were aligned and assigned to their respective genotypes by phylogenetic analysis as described previously (23). Sequences belonging to the officially extinct lineages (B1, B2, D1, E, F, and G1), as well as those belonging to genotype C1, which was last detected 10 years ago (2), were removed from the data set. Clade- and genotype-specific nucleotides were identified among the remaining 613 sequences. The number of sequences available for the different genetic groups ranged between 4 (D9 and G3) and 268 (clade D). Figure 2 shows that for most clades at least one nucleotide exists, which was not found in strains of other clades. The nucleotide A1321 was found in all but 1 (2.8%) clade A virus and in 4 (0.7%) of 594 viruses of other clades. The clade G-specific nucleotide (A1486) was also present in one D4 sequence. With the exception of D2, each genotype carried a characteristic nucleotide that was absent in any other genotype of the same clade. The D2-specific nucleotide (A1482) was also found in four viruses belonging to D4 or D6.

Primer design. Both cladetyping and genotyping reactions were based on nucleotide-specific PCR with type-specific sense primers and a common antisense primer (nt 1649 to 1629). Specificity resulted from the type-specific nucleotides of Fig. 2 incorporated as a 3′-terminal base into the respective primers. Under appropriate experimental conditions, efficient primer elongation is dependent on the matching nucleotide at the 3′ end (15). PCR conditions were optimized to enhance the inhibitory effect of a mismatch at this position. Specificity was considerably increased by using short annealing and elongation times (10 s) and a relatively high annealing temperature (66°C) (data not shown). Optimal conditions slightly differed for most primers. For instance, a high thermal stability (high GC content) at the 3′-terminal part of a primer reduced the effect of a mismatch at the 3′ end. To comply with the multiplex format, primer lengths and concentrations were adapted to a unique annealing temperature (66°C).

Fragment length and therefore the position of type-specific nucleotides within the HVR was critical to ensure proper size and type differentiation by agarose gel electrophoresis. Type-specific primers were thus designed for all active clades and genotypes after the positions and specificities of different candidate nucleotides were balanced and compatibility with experimental conditions (Table 1).

Although single point mutations within the primer binding sites should not affect the specificity of the reaction, the accumulation of mismatches may influence the efficiency of ampli-

TABLE 1. Sense primer combinations in the different multiplex PCR reactions

Mix and primer	Sequence	Concn (μM)	Position[a]
Clade mix[b]			
CIA	5′-GCAATGCATACTACTGAGGACAA-3′	0.4	1299–1321
CIB	5′-CAGGACAGTCGAAGGTCAGCC-3′	0.1	1563–1583
CIC	5′-CGAGATGGGGGGGTAAGGAAGATAT-3′	0.4	1396–1420
CID_a	5′-GATCAAAGTG*A*GA*A*TGAGCTCCCA-3′	0.2	1374–1397
CID_b	5′-GATCAAAGTG*A*GA*A*TGAGCT*A*CCA-3′	0.4	1374–1397
CID_c	5′-GATCAAAGTG*GG*AG*T*GAGCT*A*CCA-3′	0.2	1374–1397
CIG	5′-CCGGGCACAGCAGAGCAAA-3′	0.1	1468–1486
CIH	5′-CATTGACACTGCATCGGAGTA-3′	0.4	1529–1549
Genotype B Mix			
GrB3.1	5′-ACAGTCGAAGGTCAGCCGAT-3′	0.4	1567–1586
GrB3.2	5′-AGGACAGGAGGGTCAAACAGG-3′	0.4	1414–1434
Genotype D_I Mix			
GeD2	5′-GAGAAACCGGGTCCAGCAGAA-3′	0.2	1462–1482
GeD4	5′-CCCAGACAAGCCCAAGTGTCATTTA-3′	0.4	1341–1365
GeD6	5′-CCTAGACATTGACACTGCATCGGAGA-3′	0.4	1523–1548
GeD9	5′-GTCAAACAGAGTCGGGGAGAAGCA-3′	0.4	1425–1448
MVN	5′-CTGCAAGCCATGGCAGGAATC-3′	0.2	1599–1619
Genotype D_{II} Mix			
GeD3	5′-GCCCATCCTCCAACCAGCATG-3′	0.4	1500–1520
GeD5	5′-GGTATCACTGCCGAGGATGCG-3′	0.2	1260–1280
GeD7	5′-CCAAGATCTGCAGGACAGCCGAC-3′	0.2	1553–1575
GeD8	5′-GGGAGAAGCCAGGGAGAGCA-3′	0.4	1439–1458
MVN	5′-CTGCAAGCCATGGCAGGAATC-3′	0.4	1599–1619
Genotype G Mix			
GeG2	5′-GCAAATGATGCGAGAGCTGCTG-3′	0.4	1482–1503
GeG3	5′-CGGGATTGGGGGGTAAGGAAGATAAGAA-3′	0.4	1396–1423
Genotype H Mix			
GeH1	5′-CCAGGCAAGCCCAAGTCTCATTTT-3′	0.4	1342–1365
GeH2	5′-CTACAGAGAAACCGGGCTCAA-3′	0.4	1457–1477

[a] Nucleotide positions are given according to the assignments of Mori et al. (11).
[b] Degenerate positions for clade D primers are shown in italics.

fication depending on their relative positions. Some mutations were found relatively frequently within the clade D primer binding site. C1394 was present on 90% of all D5 strains, and all of the more recently detected D7 strains had two mutations in the primer region: G1384 and G1387. To compensate for this sequence variability, three similar primers (D_a, D_b, and D_c), with nucleotides matching known sequences at each of the three different sites, were combined in the cladetyping multiplex PCR (Table 1).

Cladetyping and genotyping. RT-PCR products of the complete HVR from strains of the different genotypes were first subjected to the cladetyping multiplex PCR containing type-specific sense and the common antisense primer revCG to identify the clade of the virus. Figure 3 shows that strains of each clade generated a single PCR product of the expected characteristic size, irrespective of the genotype to which the strain belonged within that clade. For instance, all D2-D9 genotypes gave a PCR product of the same length (277 bp), and no strain of another genotype gave a similar fragment. The fragment mix lanes show that PCR products of all clades can be clearly differentiated.

Depending on the clade identified, the corresponding genotyping multiplex PCR was used to assign the virus to its genotype (Fig. 3). Analysis of clade A and C strains was limited to the cladetyping reaction. Clade A consists of a single genotype, and only a single C genotype (C2) seemed to be active during the past 10 years (2). Two variants, B3.1 and B3.2, of the only active B genotype were distinguished by genotype B mix (Fig. 3). PCR products with a characteristic length were obtained for genotypes G2 and G3, as well as H1 and H2, with genotype G and genotype H mixes, respectively (Fig. 3). When all genotype D-specific primers were combined in a single multiplex PCR, the size resolution in the agarose gel was insufficient. Therefore, genotyping of clade D strains was split into two multiplex reactions: D_I containing primers GeD2, GeD4, GeD6, and GeD9 and D_{II} with the primers GeD3, GeD5, GeD7, and GeD8. All clade D templates were analyzed by both reactions. Thus, the specific primer of a given D genotype is present in only one of the two reaction mixtures. To obtain a single unequivocal band also in the irrelevant PCR mix, an additional sense primer (MVN), giving a 57-bp fragment with all genotypes, was included in both reaction mixtures D_I and D_{II} (Table 1). In some cases this fragment was also produced, together with the genotype-specific PCR product in the relevant mix (Fig. 3).

DISCUSSION

Type-specific nucleotides have been exploited to develop simple PCR-based assays to genotype viruses such as hepatitis B and C virus or Norwalk-like virus (8, 14, 16, 27). The robust-

	A [36]	B3.1 [90]	B3.2 [80]	C2 [51]	D2 [6]	D3 [48]	D4 [48]	D5 [64]	D6 [66]	D7 [20]	D8 [12]	D9 [4]	G2 [7]	G3 [4]	H1 [58]	H2 [19]
mutation	Clade specific mutations															
A1321	97.2	1.1		2.0	16.7		2.1									
C1583		100.0	100.0													
T1420				100.0												
A1397					83.3	100.0	100.0	100.0	98.5	100.0	91.7	100.0				
A1486							2.1						100.0	100.0		
A1549															100.0	100.0
mutation	Genotype specific mutations															
T1586		100.0					2.1								3.4	
G1434			98.8			4.2	12.5			55.0						
A1482					100.0		6.3		1.5							
G1520						100.0										
A1365							100.0									
G1280			51.3					100.0								
A1548									100.0							
C1575										100.0						
A1458	2.8										100.0					68.4
A1448												100.0				
G1503													100.0			
A1423											8.3			100.0		
T1365															100.0	
A1477																100.0

FIG. 2. Frequency (percent) of type-specific nucleotides in the HVR of 613 MV strains. Shaded boxes correspond to the frequency of the type-specific nucleotides within the clade or genotype. Numbers of strains per genotype are indicated within brackets. Empty boxes correspond to 0%. Nucleotide positions are assigned according to the method of Mori et al. (11).

ness of these assays depends on a careful selection of suitable nucleotide differences between the different types. Although single nucleotide differences could be selected from any part of the viral genome, for most MV strains only the HVR of the nucleoprotein can be retrieved from sequence databases. Nucleotides that were found consistently in strains of a given clade or genotype, irrespective of time of isolation and geographic origin, are likely to be conserved also in the future. For most clades or genotypes a reliable number (19 to 268) of sequences was available, whereas the genotypes D2, D8, D9, G2, and G3 were represented with only few sequences (Fig. 2). The upper limit of sensitivity of the assay corresponds to the prevalence of selected type-specific nucleotides within their clades or genotypes. Among the 613 viruses analyzed here a total of 5 (0.8%) strains did not contain their type-specific nucleotide: clade A (one strain), genotype B3.2 (one strain), and clade D (three strains). The upper limit of specificity depends on the frequency of a type-specific nucleotide to occur also in another clade or genotype. This was the case for nine (1.4%) strains, corresponding to a theoretical specificity of 98.6%. The resulting positive and negative predictive values of the assay are 99.2 and 98.6%, respectively, when the results of both cladetyping and genotyping PCR are considered (Fig. 2). Strains giving no or several bands, due to missing type-specific nucleotides or additional nucleotides specific for a different type, must be sequenced for further characterization.

Normally MV genotyping relies on RT-PCR, followed by a nested PCR, which generates the template for the sequencing reaction. Here, the sequencing reaction is replaced by two consecutive multiplex PCRs: one to identify the clade, followed by a second one to identify the corresponding genotype. PCR fragments are analyzed by conventional agarose gel electrophoresis and visual comparison of fragment size with standards, replacing complex phylogenetic analyses. Theoretically, the cladetyping and all five genotyping multiplex PCRs could be performed in parallel, since the instrumental conditions are identical. However, in this case only the genotyping results corresponding to the identified clade must be considered, since among the known sequences some genotype-specific nucleotides are also found in strains belonging to a different clade (Table 1).

Mutations in the primer region could potentially affect the efficiency of the PCR amplification and may lead to false-negative results. Therefore, sequence variability within the complete primer region was carefully analyzed. Three homologous D-specific primers (D_a, D_b, and D_c; see Table 1) were included in the clade mix to compensate for sequence variability in the clade D primer region. For the remaining types, only little variability was found in the primer region. Only single mutations were found in the different primer binding sites, except in 4 B3 strains (two mutations). If all mutations in the primer binding region would affect the assay the specificity would be unaffected but the theoretical sensitivity would be reduced to 86.4%. However, only mutations located in the binding site of the 3′-terminal primer part impaired the formation of the corresponding PCR product under the experimental conditions recommended here. Such a mutation may interfere with both primer annealing and extension. Figure 4 shows that, among all mutations located in the primer binding regions, only a small number is found in the binding site of the 3′-terminal primer part.

Three or more different strains were tested for most genotypes. Although of the most recently identified genotypes D9,

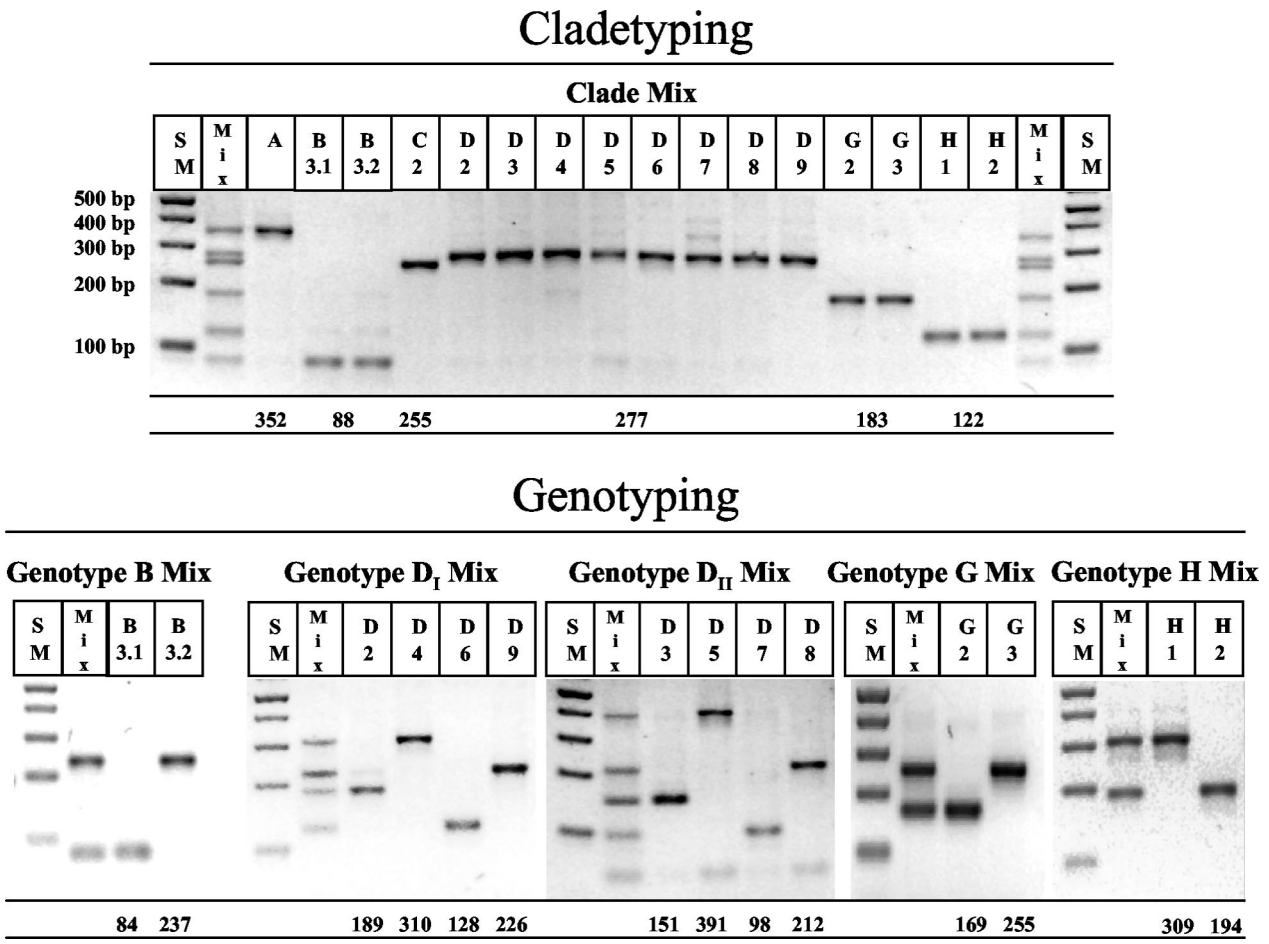

FIG. 3. Typical agarose gel electrophoresis pattern of PCR products from different MV clades and genotypes as determined by multiplex PCR. Numbers under the lanes indicate the fragment sizes in base pairs. Lanes with multiple bands correspond to fragment mix standard (Mix). SM, size marker.

G2, G3, and H2 only a single strain was available for testing, all other published sequences of these genotypes showed no mutation within the primer binding region that could interfere with amplification.

During the past 5 years a number of new genotypes have been identified, and more identifications may follow (10). Genotypes that are considered inactive and that were therefore not included in the present study (B1, B2, C1, D1, E, and F) may resurface in areas with little molecular epidemiological surveillance. For instance, clade G was considered inactive before it was detected in Indonesia in 1997, 14 years after its last detection in Berkeley, Calif. (3, 6, 19). As new genotypes are described, additional specific primers will have to be included in the multiplex PCR. Obviously, only known genotypes can be identified with the present method.

The present simplified genotyping approach is based on techniques that are commonly used in many diagnostic laboratories throughout the world. This method brings MV genotyping within reach of many more laboratories, in developing countries in particular, although the practicability in these countries needs to be confirmed. The protocol (including reagents) described here must be strictly applied by experienced lab workers to obtain reliable results. The assay is highly specific and can be adapted to different levels of characterization, and new genotypes and mutations of particular interest can be added with relative ease. However, ultimate confirmation of the results can only be obtained from sequence analysis.

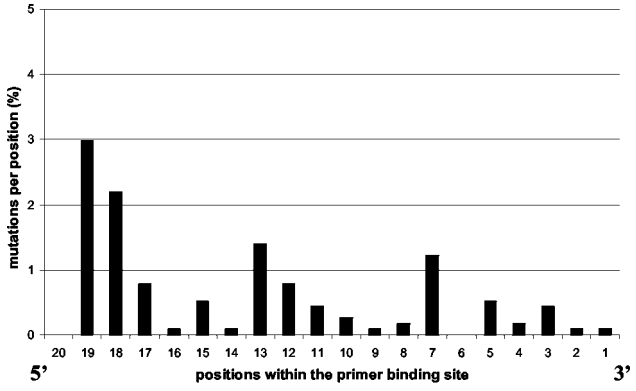

FIG. 4. Cumulated sequence variability in the different positions of the corresponding binding sites of all primers from 5′ to 3′. The data represent all mutations resulting from the comparison of all published strain sequences to their clade- and/or genotype-specific primer. Position 0 corresponds to the 3′-terminal nucleotide of the primer.

ACKNOWLEDGMENTS

This study was supported by grants from the ELSM partnership program (QLK2-CT-2001-01632) of the 5th framework of the European Commission; the Ministère des Affaires Étrangères, du Commerce Extérieur, et de la Coopération; the Ministère de la Santé; and the Centre de Recherche Public–Santé of Luxembourg. J.R.K. is supported by a BFR fellowship of the Ministère de la Recherche (Luxembourg). We also acknowledge the WHO for support and leadership.

REFERENCES

1. **Baczko, K., I. Pardowitz, B. K. Rima, and V. ter Meulen.** 1992. Constant and variable regions of measles virus proteins encoded by the nucleocapsid and phosphoprotein genes derived from lytic and persistent viruses. Virology **190:**469–474.
2. **Barrero, P. R., R. O. Zandomeni, and A. S. Mistchenko.** 2001. Measles virus circulation in Argentina: 1991–1999. Arch. Virol. **146:**815–823.
3. **Bellini, W. J., and P. A. Rota.** 1998. Genetic diversity of wild-type measles viruses: implications for global measles elimination programs. Emerg. Infect. Dis. **4:**29–35.
4. **Chibo, D., M. Riddell, M. Catton, and C. Birch.** 2002. Novel measles virus genotype, East Timor and Australia. Emerg. Infect. Dis. **8:**735–737.
5. **Chibo, D., M. Riddell, M. Catton, M. Lyon, G. Lum, and C. Birch.** 2003. Studies of measles viruses circulating in Australia between 1999 and 2001 reveals a new genotype. Virus Res. **91:**213–221.
6. **de Swart, R. L., P. M. Wertheim-van Dillen, R. S. van Binnendijk, C. P. Muller, J. Frenkel, and A. D. Osterhaus.** 2000. Measles in a Dutch hospital introduced by an immuno-compromised infant from Indonesia infected with a new virus genotype. Lancet **355:**201–202.
7. **Hanses, F., A. T. Truong, W. Ammerlaan, O. Ikusika, F. Adu, A. O. Oyefolu, S. A. Omilabu, and C. P. Muller.** 1999. Molecular epidemiology of Nigerian and Ghanaian measles virus isolates reveals a genotype circulating widely in western and central Africa. J. Gen. Virol. **80:**871–877.
8. **Kirschberg, O., C. Schuttler, R. Repp, and S. Schaefer.** 2004. A multiplex-PCR to identify hepatitis B virus-genotypes A-F. J. Clin. Virol. **29:**39–43.
9. **Kreis, S., E. Vardas, and T. Whistler.** 1997. Sequence analysis of the nucleocapsid gene of measles virus isolates from South Africa identifies a new genotype. J. Gen. Virol. **78:**1581–1587.
10. **Kremer, J. R., and C. P. Muller.** 2003. Molecular epidemiology of measles in Asia. Curr. Top. Virol. **3:**165–181.
11. **Mori, T., K. Sasaki, H. Hashimoto, and S. Makino.** 1993. Molecular cloning and complete nucleotide sequence of genomic RNA of the AIK-C strain of attenuated measles virus. Virus Genes. **7:**67–81.
12. **Mulders, M. N., and C. P. Muller.** 2002. Molecular epidemiology of measles virus, p. 237–272. *In* Thomas Leitner (ed.), The molecular epidemiology of human viruses. Kluwer Academic Publishers, Boston, Mass.
13. **Mulders, M. N., A. T. Truong, and C. P. Muller.** 2001. Monitoring of measles elimination using molecular epidemiology. Vaccine **19:**2245–2249.
14. **Naito, H., S. Hayashi, and K. Abe.** 2001. Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers. J. Clin. Microbiol. **39:**362–364.
15. **Newton, C. R., A. Graham, L. E. Heptinstall, S. J. Powell, C. Summers, N. Kalsheker, J. C. Smith, and A. F. Markham.** 1989. Analysis of any point mutation in DNA: the amplification refractory mutation system (ARMS). Nucleic Acids Res. **17:**2503–2516.
16. **Ohno, O., M. Mizokami, R. R. Wu, M. G. Saleh, K. Ohba, E. Orito, M. Mukaide, R. Williams, and J. Y. Lau.** 1997. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J. Clin. Microbiol. **35:**201–207.
17. **Rota, J. S., J. L. Heath, P. A. Rota, G. E. King, M. L. Celma, J. Carabana, R. Fernandez-Munoz, D. Brown, L. Jin, and W. J. Bellini.** 1996. Molecular epidemiology of measles virus: identification of pathways of transmission and implications for measles elimination. J. Infect. Dis. **173:**32–37.
18. **Rota, P. A., A. E. Bloom, J. A. Vanchiere, and W. J. Bellini.** 1994. Evolution of the nucleoprotein and matrix genes of wild-type strains of measles virus isolated from recent epidemics. Virology **198:**724–730.
19. **Rota, P. A., S. Liffick, S. Rosenthal, B. Heriyanto, and K. B. Chua.** 2000. Measles genotype G2 in Indonesia and Malaysia. Lancet **355:**1557–1558.
20. **Samuel, D., S. Beard, H. Yang, N. Saunders, and L. Jin.** 2003. Genotyping of measles and mumps virus strains using amplification refractory mutation system analysis combined with enzyme immunoassay: a simple method for outbreak investigations. J. Med. Virol. **69:**279–285.
21. **Santibanez, S., A. Tischer, A. Heider, A. Siedler, and H. Hengel.** 2002. Rapid replacement of endemic measles virus genotypes. J. Gen. Virol. **83:**2699–2708.
22. **Takahashi, M., T. Nakayama, Y. Kashiwagi, T. Takami, S. Sonoda, T. Yamanaka, H. Ochiai, T. Ihara, and T. Tajima.** 2000. Single genotype of measles virus is dominant, whereas several genotypes of mumps virus are co-circulating. J. Med. Virol. **62:**278–285.
23. **Truong, A. T., S. Kreis, W. Ammerlaan, H. K. Hartter, F. Adu, S. A. Omilabu, A. O. Oyefolu, G. A. Berbers, and C. P. Muller.** 1999. Genotypic and antigenic characterization of hemagglutinin proteins of African measles virus isolates. Virus Res. **62:**89–95.
24. **Truong, A. T., M. N. Mulders, D. C. Gautam, W. Ammerlaan, R. L. de Swart, C. C. King, A. D. Osterhaus, and C. P. Muller.** 2001. Genetic analysis of Asian measles virus strains: new endemic genotype in Nepal. Virus Res. **76:**71–78.
25. **World Health Organization.** 2003. Update of the nomenclature for describing the genetic characteristics of wild-type measles viruses: new genotypes and reference strains. Wkly. Epidemiol. Rec. **78:**229–232.
26. **Xu, W., A. Tamin, J. S. Rota, L. Zhang, W. J. Bellini, and P. A. Rota.** 1998. New genetic group of measles virus isolated in the People's Republic of China. Virus Res. **54:**147–156.
27. **Yuen, L. K., M. G. Catton, B. J. Cox, P. J. Wright, and J. A. Marshall.** 2001. Heminested multiplex reverse transcription-PCR for detection and differentiation of Norwalk-like virus genogroups 1 and 2 in fecal samples. J. Clin. Microbiol. **39:**2690–2694.