Reproducibility of *Bordetella pertussis* Genomic DNA Fragments Generated by *Xba*I Restriction and Resolved by Pulsed-Field Gel Electrophoresis

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Received 2 April 2001/Returned for modification 19 June 2001/Accepted 10 December 2001

The intra- and interlaboratory variabilities of the molecular size measurements of each DNA fragment contributing to three pulsed-field gel electrophoresis (PFGE) profiles were assessed, as were the reproducibilities of the entire PFGE profiles for three *Bordetella pertussis* strains. The major source of variability within a laboratory occurred between subcultures rather than within gels or between gels. Each PFGE profile was generated reproducibly and was objectively defined by the molecular sizes of its composite fragments. A strain or profile most suitable for use as an internal reference standard was identified.

The introduction of pertussis vaccination in the United States in the mid-1940s dramatically reduced the rates of morbidity and mortality from pertussis and fundamentally altered the epidemiology of this disease. More modest changes in pertussis epidemiology have been noted recently, including the increased incidence reported beginning in the mid-1980s in the highly vaccinated U.S. population, the emergence of macrolide resistance in *Bordetella pertussis* in the early 1990s, and the increased number of reported cases in persons aged 10 to 19 years beginning in the mid-1990s (6, 7, 9, 11, 14). The *B. pertussis* population in the United States and elsewhere has now been shown to be dynamic, and shifts in the circulating population may reflect changes in pertussis epidemiology (4, 12, 15, 17, 22). Consequently, it has become critical to monitor the *B. pertussis* population for changes that might have epidemiologic relevance or that might affect recent and future prevention strategies.

Pulsed-field gel electrophoresis (PFGE) subtyping of *B. pertussis* isolates has been successfully used in epidemiologic investigations to identify outbreak-associated isolates and to monitor transmission (2, 4, 5, 8, 12, 15, 21). Recent studies have refined the PFGE conditions for optimal discrimination of the profiles generated by use of the restriction endonuclease *Xba*I (10, 12). Consequently, the second enzyme (*Spe*I) whose use was previously recommended (2, 16) for the resolution of slight differences between the very closely related profiles generated by use of *Xba*I is only rarely needed.

To maximize their epidemiologic impacts, the PFGE profiles generated in different laboratories must be comparable (1, 18).

However, the comparison of *B. pertussis* PFGE subtypes from different laboratories is limited because the reproducibilities of the DNA fragment molecular sizes and of the PFGE profiles are unknown. In addition, no internal molecular size standard has been widely accepted for use for the transformation of the migration distances of the DNA fragments into their respective molecular sizes. The goals of this study were to determine the variabilities of the molecular size measurements among the *B. pertussis* DNA fragments generated by PFGE, to evaluate the reproducibilities of the profiles generated in different laboratories, and to identify the PFGE profile most suitable for use as an internal molecular size standard.

MATERIALS AND METHODS

We selected three *B. pertussis* strains, strains A556, A560, and A639, that yielded PFGE profiles CYXXI-004, CYXXI-002, and CYXXI-006, respectively, for this study. Profile identifiers were designated by the letters CY (for *Bordetella*), X (for *pertussis*), and XI (for the restriction endonuclease *Xba*I), followed by three number fields representing the PFGE profile. We selected these strains for several of their PFGE profile properties, including their prevalences among isolates currently circulating in the United States, the ranges of their DNA fragment molecular sizes being inclusive of those commonly encountered, the prevalences and discriminatory powers of their DNA fragments among all recognized profiles, and the larger number of DNA fragments that appear in the central compression zone (3). This zone consists of a small area common to the majority of profiles where it is difficult to differentiate the multiple DNA fragments that it contains.

The laboratories participating in this study included the Department of Clinical Microbiology, Arizona Department of Health Services, Phoenix; Epidemiologic Investigations Laboratory, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Microbiology Laboratory, Minnesota Department of Health, Minneapolis; State Laboratory Institute, Diagnostic Laboratories, Massachusetts State Public Health Laboratory, Jamaica Plain, Mass.; and the Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada. The five laboratories were randomly assigned alphabetical identifiers and are referred to as laboratories A, B, C, D, and E. The participating laboratories subcultured the three assay strains twice weekly for 3

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FIG. 1. PFGE of DNA fragments from assay strains generated by restriction of genomic DNA with *Xba*I. The molecular size (molecular weight) ranges of discriminatory fragments are distinguished by the DNA fragments within the dotted lines.

frweeks; the first subculture was used to harvest growth for PFGE analysis, and the second subculture was used to maintain culture continuity. Test strains were grown on charcoal agar (Oxoid, Unipath, Ltd., Basingstoke, England) with 10% defibrinated horse blood (Lampire Biological Laboratories, Pipersville, Pa.) under ambient air and high humidity for 3 to 4 days. Laboratories B, C, D, and E evaluated all three test strains; laboratory A evaluated only strains A560 and A639. Laboratories B and D used a power source of the same model. Laboratories C and E also used a power source of the same model, but the power source differed from the one common to laboratories B and D. Only laboratories A and C used a single gel box and power source exclusively.

The test strains were evaluated by methods based on the methods of Gautum (10), as modified by others (12). Briefly, cells from the test strains were suspended in buffer in duplicate and the suspensions were added to molten agarose to form duplicate sets of plugs. The plugs were treated to lyse the cells and purify the genomic DNA, which was subsequently digested with *Xba*I (Roche Molecular Biochemical, Indianapolis, Ind.). Each DNA digest was randomly applied to four replicate lanes of an agarose gel except lanes 1, 8, and 15. These three lanes contained the reference standard of concatemers of 48.5-kb phage lambda DNA (Roche Molecular Biochemical) used to transform *B. pertussis* DNA fragment migration distances to molecular sizes. Thus, the profile for each strain was replicated eight times over two gels from duplicate restriction digests of DNA from a single subculture. This procedure was repeated for three subcultures, generating 24 replicate lanes per assay strain. All reagents and enzymes used were from the same manufacturer's stock.

We determined the intra- and interlaboratory variabilities of the molecular size measurements of the DNA fragments obtained as described. We also compared the reproducibilities of the PFGE profiles between laboratories. The participating laboratories sent unmodified computer images (tagged image file format) of their gels to the Centers for Disease Control and Prevention for analysis. We used Molecular Analyst (Bio-Rad, Hercules, Calif.) software to normalize the DNA fragment migration distances relative to those of the con-

catemers of the 48.5-kb phage lambda DNA and to transform normalized migration distances to molecular sizes. After the molecular sizes were defined for each DNA fragment, the data were transformed into a statistically acceptable format for analysis with SAS software (SAS Institute, Inc., Cary, N.C.).

We expressed relative molecular size measurement variability as a coefficient of variation [CV (in percent); CV = (standard deviation/mean) $\times$ 100] (19, 20). The significance of differences between the arithmetic means was determined by a *t* test to analyze differences between pairs (19, 20). In addition, a mixed-model analysis of variance procedure was used to examine differences in arithmetic mean molecular size measurements for each DNA fragment separately for each assay strain. This procedure accounted for the repeated measures design of the study (four replicate strain digests were included on each of two gels, and this was repeated three times for a total of 24 replicate profiles). This analysis also took into account the inherent colinearity among molecular size measurements of a gel. Each laboratory site represented the fixed-effect portion of the study; the lanes on each gel and the gels themselves accounted for the random effect. A compound symmetric correlation matrix was used to model the correlation among DNA fragment lengths. The SAS MIXED procedure (SAS Institute, Inc.) was used to analyze the results. Appropriate linear contrasts were formed within the procedure to examine mean differences among gels and among study sites. The molecular sizes of adjacent fragments were compared by a Wilcoxon rank sum test.

RESULTS

B. pertussis strains A556, A560, and A639 produced PFGE profiles designated CYXXI-004, CYXXI-002, and CYXXI-006, respectively (Fig. 1). Fourteen DNA fragments ranging in molecular size from 123 to 395 kb and previously shown to

TABLE 1. Mean and range DNA fragment molecular sizes determined by PFGE analysis of three *B. pertussis* strains in five laboratories

DNA fragment	Molecular size (kb) of DNA fragments of <i>B. pertussis</i> of PFGE profile (strain):					
	CYXXI-004 (A556)		CYXXI-002 (A560)		CYXXI-006 (A639)	
	Mean	Range	Mean	Range	Mean	Range
1	393.65	392.56–394.73				
2					363.54	356.66–367.24
3			321.06	319.12–322.81		
4	296.51	294.74–297.77	297.90	293.21–300.45	297.03	293.22–299.91
5			264.44	262.24–267.18	267.63	266.29–270.07
6	255.76	254.19–257.85	255.40	253.80–259.33	256.63	254.78–259.52
7	243.35	242.40–244.72				
8			225.69	223.52–227.59		
9	211.84	211.52–212.70	211.51	209.51–212.73	212.07	210.69–213.34
10	190.75	188.92–192.13	188.28	186.95–189.09	188.71	187.58–189.54
11	177.75	175.57–179.16				
12	147.98	145.63–149.90	148.52	148.07–149.43	148.61	147.11–150.74
13	130.63	128.59–132.50				
14	124.97	123.08–126.22	125.14	123.44–126.49	124.8	122.99–126.67

discriminate among the profiles from epidemiologically unrelated isolates contributed to the differentiation of PFGE profiles (2). Six fragments (fragments 4, 6, 9, 10, 12, and 14) were common to all three profiles, and one fragment (fragment 5) was common to two profiles (Table 1). All others (fragments 1, 2, 3, 7, 8, 11, and 13) were unique to their respective profiles. Profiles CYXXI-002, CYXXI-004, and CYXXI-006 comprised 10, 9, and 8 DNA fragments, respectively.

Since the range of molecular sizes of the fragments was different for each profile and varied as much as 272 kb within a profile and since the true molecular sizes were unknown, we expressed variability as a CV in percent. We first examined the variability in the mean molecular size for each DNA fragment replicated ($n = 4$) within a gel for each laboratory (Table 2). The CVs were $<2.3\%$, regardless of the fragment molecular size, the strain or profile, or the laboratory. We also used CV to express the variabilities in fragment mean molecular sizes between gels ($n = 24$) in each laboratory (Table 3). We found that all values were $<4.0\%$ and that most laboratories recorded slightly higher CVs for fragments with molecular sizes <140 kb compared to those for larger fragments, regardless of the strain or profile. Two laboratories (laboratories A and E) uniformly reported lower CVs ($<2.0\%$) than the other three laboratories. These two laboratories used different models of power supply, and only one used a single gel box and power supply exclusively.

Our study also measured the variabilities in the molecular size of each fragment between the three cultures of each test strain. A mean molecular size was calculated for each fragment from every subculture (data not shown), and the significance of the differences between the means from different subcultures was determined. Significant differences in the mean molecular sizes of many fragments from different cultures were observed (139 of 375 comparisons). Comparatively fewer significant differences in mean molecular sizes were observed at laboratories C and E ($\sim 20\%$) compared to the proportions of significant differences in mean molecular sizes observed at laboratories A, B, and D ($>85\%$). Only one of the laboratories producing fewer significant differences used a single gel box and power supply exclusively, but the power supply was common to the other laboratories as well. Also, fewer differences were noted

between cultures 2 and 3 (35%) versus cultures 1 and 2 (53%) and cultures 1 and 3 (42%) when the mean molecular sizes of all fragments from all three strains were compared. The number of significant differences associated with a given test strain was consistent at approximately 37%.

We determined the interlaboratory variabilities by calculating the CVs of the mean molecular sizes for every DNA fragment replicated ($n = 24$) in each laboratory ($n = 5$) (Table 4). The lowest CV was 0.26% for fragment 1, and the highest was 1.19% for fragment 13, both from a strain with profile CYXXI-004. We noted that fragments <140 kb tended to have higher CVs. We used the ranges of the mean molecular sizes of the fragments comprising each profile to measure the reproducibilities of the three test profiles between laboratories (Table 1). The mean molecular size ranges of all fragments were small enough not to coincide with the ranges of molecular sizes of adjacent fragments. Moreover, the ranges of molecular sizes of analogous fragments in different profiles were coincident only to each other and not to those of adjacent fragments in the other profile(s). Consequently, the respective profiles were highly reproducible between laboratories and no profile was more reliable than another.

As a consequence of the last result, we recommend the use of strain A639 with profile CYXXI-006 as the reference standard. This profile includes fragments with sizes throughout the general molecular size range of discriminatory fragments from 125 to 450 kb. This profile has the added advantage of four fragments in the molecular size range of 250 to 400 kb, where it is most difficult to discriminate between adjacent fragments.

DISCUSSION

In recent years, PFGE of endonuclease-generated fragments of *B. pertussis* chromosomal DNA has been used for multiple purposes, but it has most frequently been used to subtype isolates for epidemiologic studies (2, 4, 5, 8, 12, 13, 15, 21, 22). This technology was also recently recommended for use for epidemiologic applications by an international panel of subtyping experts (16). However, no systematic standardization of the methodology has been performed, nor has the intra- or interlaboratory comparability of the procedure been assessed,

TABLE 2. CVs of DNA fragment molecular sizes determined by PFGE analysis of three *B. pertussis* strains in five laboratories

Test strain (DNA fragment)	CV (%) for the following participating laboratory and gel ^a :																													
	A						B						C						D						E					
	Gel 1	Gel 2	Gel 3	Gel 4	Gel 5	Gel 6	Gel 1	Gel 2	Gel 3	Gel 4	Gel 5	Gel 6	Gel 1	Gel 2	Gel 3	Gel 4	Gel 5	Gel 6	Gel 1	Gel 2	Gel 3	Gel 4	Gel 5	Gel 6						
A556 (1)	0.55	0.43	0.88	0.95	0.33	1.77	0.88	0.36	1.02	1.24	0.68	0.35	0.90	0.7	1.68	0.69	1.02	1.08	1.06	0.35	0.67	0.58	0.35	1.07						
A556 (4)	0.38	0.52	1.19	0.94	0.81	0.54	1.19	0.00	0.84	1.35	0.98	0.38	0.96	0.32	1.37	0.61	1.09	0.81		0.80	0.55	0.87	0.78							
A556 (6)	0.48	0.50	1.06	0.52	0.26	0.27	0.85	0.38	0.99	1.51	1.26	0.38	0.80	0.52	1.38	0.80	1.11	0.52	1.03	0.40	0.34	0.97	1.59							
A556 (7)	0.23	0.53	1.17	0.58	0.45	0.53	0.86	0.33	1.13	1.57	0.94	0.38	1.09	0.61	1.70	0.91	0.82	0.53	1.40	0.35	0.40	0.35	0.69	1.85						
A556 (9)	0.29	0.56	1.16	0.68	0.48	0.97	1.27	0.34	1.18	1.42	0.98	0.35	0.64	0.55	1.48	0.96	1.31	0.34	1.33	0.70	0.37	1.03	1.12							
A556 (10)	0.40	0.39	1.05	0.47	0.53	1.07	1.25	0.36	0.93	1.59	1.37	0.69	0.90	0.81	1.57	0.69	1.24	0.69	1.32	0.73	0.00	0.44	1.31	1.34						
A556 (11)	0.49	0.81	0.55	0.66	0.32	1.11	1.32	0.37	1.28	1.28	1.30	0.43	1.24	0.37	1.79	1.07	1.29	0.48	1.18	0.65	0.40	0.40	1.00	1.67						
A556 (12)	0.46	0.94	1.07	1.06	0.69	1.19	1.07	0.41	1.25	1.59	1.45	0.42	1.38	0.67	1.84	1.20	1.45	0.82	0.83	0.84	0.72	1.50	2.05							
A556 (13)	0.93	0.93	1.08	0.86	0.54	0.84	0.99	0.57	2.04	2.16	1.35	0.68	1.36	0.43	1.33	0.46	1.60	1.38	1.03	0.61	0.45	0.86	1.77	2.07						
A556 (14)	0.69	1.29	0.92	0.39	0.46	1.16	0.61	0.59	1.95	1.75	0.78	0.59	1.65	0.46	1.65	0.55	1.65	0.91	0.98	0.48	1.00	1.65	1.49							
A560 (3)	0.35	0.89	0.81	0.38	0.38	0.13	0.54	0.38	0.63	1.00	0.00	0.33	0.53	0.84	0.81	0.92	1.84	1.95	1.27	0.64	0.34	0.34	0.87	0.34						
A560 (4)	0.43	0.64	0.74	0.13	0.67	0.34	0.33	0.38	0.76	1.14	0.00	0.33	0.37	0.91	1.10	0.63	2.02	1.41	0.95	0.34	0.34	0.39	0.65	0.55						
A560 (5)	0.13	0.44	0.46	0.93	0.56	0.81	0.26	0.95	0.52	0.64	0.00	0.63	0.00	0.32	0.84	1.37	0.52	1.58	1.24	0.34	5.38	0.34	0.56	0.65						
A560 (6)	0.35	0.59	0.68	0.77	0.14	0.91	0.35	1.00	0.81	0.26	0.68	0.52	0.32	0.56	0.97	1.34	0.16	0.27	0.54	0.60	1.22	0.32	1.33	1.38						
A560 (8)	0.29	0.33	0.55	0.98	0.28	0.99	0.29	0.79	1.21	0.54	0.65	0.57	0.56	0.00	1.17	1.01	0.00	0.34	0.62	0.76	1.13	0.66	1.74	1.08						
A560 (9)	0.29	0.34	0.76	0.59	0.44	0.56	0.34	0.76	1.30	0.77	0.88	0.57	0.35	0.00	1.04	0.89	0.35	0.4	0.33	0.87	1.29	0.68	2.11	1.46						
A560 (10)	0.56	0.31	1.06	0.62	0.63	0.51	0.51	1.10	1.40	0.63	0.89	0.60	1.10	0.41	1.26	1.09	0.00	0.42	0.57	0.58	1.24	0.72	1.82	1.81						
A560 (12)	0.36	0.59	0.69	1.25	1.09	0.60	0.69	1.23	1.29	1.25	0.93	0.92	1.62	0.78	0.97	0.48	0.42	0.48	0.66	0.80	1.45	1.20	2.13	1.45						
A560 (14)	0.40	0.66	0.40	1.52	0.00	1.80	0.74	1.37	1.80	0.46	0.76	1.14	1.60	0.52	1.19	0.88	0.46	0.89	0.45	0.96	1.18	1.42	2.52	2.52						
A639 (2)	0.7	0.31	0.27	0.81	0.52	1.17	0.14	0.16	1.40	0.73	0.00	0.42	0.66	0.35	0.87	0.66	0.34	0.00	0.66	0.67	1.69	0.66	1.49	1.32						
A639 (4)	0.79	0.31	0.61	0.79	0.55	0.92	0.40	0.52	0.88	0.77	0.27	0.00	0.84	0.38	0.84	0.65	0.33	0.00	0.61	0.64	0.61	0.32	1.69	1.32						
A639 (5)	0.49	0.62	0.31	1.05	0.26	0.94	0.27	0.50	1.05	0.90	0.31	0.54	0.82	0.62	1.13	0.62	0.33	0.33	0.82	0.80	0.82	0.32	1.68	1.21						
A639 (6)	0.46	0.54	0.26	0.95	0.26	0.44	0.26	0.57	1.16	0.55	0.61	0.69	0.56	0.94	1.35	0.72	0.46	0.31	1.00	0.66	0.78	0.32	1.71	0.74						
A639 (9)	0.56	0.68	1.00	0.75	0.48	0.96	0.29	0.59	1.10	0.34	0.29	0.34	0.67	0.56	1.33	0.56	0.80	0.35	0.77	0.38	0.96	0.00	1.94	0.65						
A639 (10)	0.31	0.72	0.80	1.06	0.51	1.34	0.31	0.47	1.24	0.88	0.31	0.36	0.92	0.42	1.26	0.59	0.70	0.70	0.00	0.91	1.03	0.42	1.97	0.94						
A639 (12)	0.00	0.72	0.36	2.12	0.91	1.09	0.59	0.59	0.72	0.80	0.68	0.42	0.81	1.24	1.43	0.68	1.26	1.18	0.46	1.03	1.27	1.19	2.23	1.56						
A639 (14)	0.40	0.79	1.04	2.10	1.04	0.66	0.46	1.33	1.72	0.40	0.40	0.84	0.90	0.87	1.07	0.75	1.08	0.46	0.86	1.76	0.55	1.35	2.28	2.00						

^a Blank cells indicate no data.

TABLE 3. CVs of DNA fragment molecular sizes determined between gels by PFGE analysis of three *B. pertussis* strains in five laboratories

Test strain and DNA fragment	Results for the following participating laboratory ^a :									
	A		B		C		D		E	
	Mean size (kb)	CV (%)	Mean size (kb)	CV (%)	Mean size (kb)	CV (%)	Mean size (kb)	CV (%)	Mean size (kb)	CV (%)
A556										
1			394	1.27	393	2.06	393	1.77	395	0.69
4			298	1.58	298	1.78	296	1.94	295	0.54
6			258	1.51	254	1.56	255	2.09	256	0.97
7			245	1.68	242	1.64	243	2.14	243	1.00
9			213	2.24	212	1.82	212	2.48	211	0.92
10			191	2.68	192	1.93	191	2.94	189	0.92
11			178	2.78	179	1.82	178	2.93	176	1.17
12			148	3.08	149	1.68	150	3.47	146	1.72
13			131	3.25	131	1.92	133	3.39	129	1.49
14			125	2.93	125	2.10	126	3.60	123	1.57
A560										
3			322	1.47	320	1.64	319	1.47	323	0.8
4	293	0.88	299	1.54	299	1.48	298	1.48	301	0.89
5	262	0.69	267	1.47	264	1.54	264	1.53	264	1.64
6	253	0.68	259	1.64	254	1.58	256	1.61	255	0.28
8	224	0.47	228	1.8	225	1.44	226	1.84	226	0.82
9	210	0.63	213	2.11	212	1.43	212	2.23	212	1.04
10	187	0.89	189	2.46	189	1.66	189	2.40	188	1.05
12	148	0.9	148	2.71	149	1.75	149	3.14	147	1.43
14	123	0.75	126	2.91	126	2.08	127	3.66	124	1.57
A639										
2	357	0.8	365	1.26	363	1.75	365	1.80	367	0.32
4	293	0.73	300	1.38	297	1.54	297	1.76	298	0.97
5	266	1.11	270	1.57	267	1.73	268	2.10	272	1.21
6	255	0.82	260	1.48	255	1.56	257	2.08	257	1.13
9	211	0.53	213	2	212	1.5	213	2.64	212	1.18
10	188	0.5	190	2.46	189	1.58	190	2.79	188	1.35
12	148	0.69	149	2.65	148	1.82	151	3.53	147	1.77
14	125	0.88	125	2.68	124	2.13	127	3.98	123	1.93

^a Blank cells indicate no data.

although these are critical to the dissemination of the technology and to the comparison of results from different laboratories. In this study, we evaluated a standardized procedure for determination of the comparability of results within and between laboratories. Our PFGE conditions maximally resolved DNA fragments 100 to 500 kb because these fragments were found to be more discriminatory and reproducible than other fragments (2, 4, 5, 12). Because the true molecular sizes of the relevant DNA fragments were not known, it was not possible to directly determine the accuracies of the measurements relative to established values. Consequently, we evaluated the variabilities of the molecular sizes of each fragment in three test profiles within gels, between gels, between cultures, and between laboratories. We also determined the interlaboratory reproducibilities of the three test profiles and identified a reference strain and profile for use as an internal reference in transforming DNA fragment migration distances (R_f) into molecular sizes (in kilobases).

The intra-assay variabilities of fragment molecular sizes, expressed as CVs, were uniformly low in all laboratories, implying a high degree of reproducibility within a gel, regardless of the fragment molecular size, the strain or profile, the laboratory, or the equipment used. The molecular size measurements for smaller fragments were somewhat more variable, but this difference was not significant. Similarly, we showed that the

interassay variability in the same laboratory was low between gels with the same DNA extraction of a subculture. Again, the molecular size measurements were somewhat more variable for smaller fragments, but this difference was not significant. Two laboratories uniformly reported lower variabilities than

TABLE 4. CVs of mean DNA fragment molecular sizes determined by PFGE analysis of three *B. pertussis* strains in five laboratories

DNA fragment	CV (%) for <i>B. pertussis</i> with the following PFGE profile (strain):		
	CYXXI-004 (A556)	CYXXI-002 (A560)	CYXXI-006 (A639)
1	0.26		
2			1.13
3		0.53	
4	0.49	0.93	0.83
5		0.67	0.55
6	0.60	0.96	0.75
7	0.41		
8		0.69	
9	0.43	0.57	0.54
10	0.70	0.47	0.59
11	0.83		
12	1.16	0.57	0.90
13	1.19		
14	1.02	0.98	1.07

the other three laboratories. The reason for this difference was not apparent, as the lower variability was not uniformly associated with the exclusive use of a single gel box and power supply. The additional variables inherent in preparing and loading replicate gels and electrophoresis of those gels probably accounted for the slight increase in interassay variability (between gels) compared to the intra-assay variability (within gels, between lanes) that we observed.

We also evaluated the contributions of different subcultures to the variabilities of the fragment molecular size measurements and found significant differences for 37% of the fragments for each of the three test strains. This finding may be explained by the variabilities in DNA extraction, preparation, and restriction as well as by the biological variations inherent in bacterial populations. However, significant differences were least frequent between subcultures 2 and 3, suggesting that the variation decreased with consecutive subcultures, perhaps by adaptation of the cells to the culture conditions. Thus, the major source of variability within a laboratory derives from the preparation of DNA from different cultures rather than from the use of different gel preparations or the use of different equipment. However, the standardized procedure produced PFGE profiles that were sufficiently reproducible to allow each laboratory to objectively define each of the three profiles by the molecular sizes of its composite fragments.

Profiles from different laboratories are conventionally identified by aligning fragments with an internal standard and visually comparing the relative positions of these fragments (3). However, this subjective approach is readily confounded by the multiple fragments that may occur within a small region of the profile and by fragments with highly similar molecular sizes. To determine if the limitations of this subjective approach could be overcome, we also evaluated the interlaboratory variabilities of the fragment molecular sizes. Although we evaluated only three *B. pertussis* strains, the DNA fragments comprising their respective profiles represented almost all discriminatory fragments comprising the profiles observed to date. Our results showed that all three profiles from the participating laboratories were reproducible and identifiable by their composite DNA fragments. Consequently, we will be able to objectively define most PFGE profiles by the molecular sizes of their composite fragments and compare profiling results between laboratories, thereby enhancing the epidemiologic relevance and utility of profiling data. Only one laboratory performed the gel lane alignments and transformed R_f values to molecular sizes, but our results have defined the DNA fragment length measurement variability, which is applicable to any algorithm that compares and clusters PFGE profiles.

Lastly, we identified strain A639 as the most appropriate reference standard because the DNA fragments composing its corresponding profile are frequently seen among the profiles for isolates recently circulating in the United States, the range of molecular sizes of the DNA fragments comprising this profile included the molecular sizes of most of the fragments from profiles observed to date, and this profile includes multiple DNA fragments that are difficult to resolve due to their proximity within a small central region of the profile. This strain can serve as the global reference standard upon integration of our *B. pertussis* subtyping activity into the PulseNet, the National

Subtyping Network for Foodborne Disease Surveillance. This effort will maximize our ability to monitor the PFGE profiles of *B. pertussis* strains circulating nationally and internationally for epidemiologically relevant changes.

ACKNOWLEDGMENT

We gratefully acknowledge Sharee Kuny for her diligence and technical expertise, which greatly contributed to this project.

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