



Adapting typing for national laboratory-based surveillance of methicillin-resistant

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1 **Adapting *spa* typing for national laboratory-based surveillance of methicillin-**
2 **resistant *Staphylococcus aureus***

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Abstract

Laboratory-based surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) monitors the baseline occurrence of different genotypes, and identifies strains and transmission chains responsible for outbreaks. The consequences of substituting pulsed-field gel electrophoresis (PFGE) with *spa* typing as a first-line typing method were analyzed by typing 589 strains isolated between 1997 and 2006, with a focus on both short- and long-term correspondence between the PFGE and *spa* typing results. The study, covering these ten years, included all Finnish MRSA blood isolates and representatives of the two most prevalent MRSA strains (PFGE types FIN-4 and FIN-16) in Finland. In addition, all sporadic isolates from 2006 were included. *spa* typing was more expensive, but approximately four times faster to perform than PFGE. Nearly 90% of FIN-4 and FIN-16 isolates showed consistent *spa* types, t172 and t067 respectively. *spa* typing predicted the PFGE result of the blood isolates by a Wallace coefficient of 0.9009, recognized internationally successful strains (t041, t067) to be common also in Finland, and identified a separate cluster of isolates, also related in time and place among the FIN-4 strains. Additional typing by another method was needed to provide adequate discrimination, or to characterize isolates with a newly recognized *spa* type in Finland.

Keywords

MRSA; *spa* typing; MLST; PFGE; laboratory-based surveillance

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a persistent public health problem worldwide. Currently, MRSA infection can be acquired both in healthcare institutions and in the community. In Finland, the annual number of MRSA cases has increased from 340 in 2001 to 1473 in 2004 [1] and in the year 2008, MRSA case numbers reached a level higher than ever before, at 1741 new cases [2].

Pulsed-field gel electrophoresis (PFGE) was originally developed for outbreak investigations, and its use in long-term laboratory-based surveillance can be misleading [3, 4]. Lately, the interpretation of PFGE typing results has become even more challenging as the number of profiles in the databases continues to grow. Concurrently, *spa* typing has become a widely used method to distinguish different MRSA strains. It allows unambiguous data interpretation, and therefore also international comparison [5, 6]. However, in some instances, discriminatory power of *spa* typing is not as high as that of PFGE [7-9].

To enable straightforward and comprehensive international comparability and to avoid excessive work load in the national MRSA surveillance and epidemiological investigations, we analyzed the consequences of substituting PFGE with *spa* typing as the primary method for MRSA typing in Finland. We focused both on the short- (year 2006) and long-term (years 1997-2006) correspondence between the PFGE and *spa* typing results. As study material, we selected MRSA blood isolates, sporadic MRSA isolates, and two strains, PFGE types FIN-4 and FIN-16, which are currently the most common community-associated MRSA (CA-MRSA) and healthcare-associated (HA-MRSA) strains, respectively, in Finland [10].

Materials and methods

Bacterial isolates

In Finland, clinical microbiology laboratories report all MRSA findings from clinical or screening samples to the national infectious disease register (NIDR) and multiple findings within 50 years from the same person are combined as one. Corresponding MRSA isolates are sent to the Staphylococcal Reference Laboratory at the National Institute for Health and Welfare (THL) for genotyping.

In this study a total of 589 MRSA isolates (one per person), genotyped previously by PFGE, was analyzed by *spa* typing. To analyze the short-term correspondence between the two methods, 108 FIN-4 (typically ST375, SCC*mec* IV) and 116 FIN-16 (typically ST125, SCC*mec* IA) isolates from the year 2006, as well as all 49 sporadic strain types (as determined by PFGE) from 2006 were chosen. For the FIN-4 and FIN-16 isolates, the first ten isolates of every month in 2006, if available, were chosen.

To study the long-term correspondence, three sets of isolates between the years 1997 and 2006 were chosen: i) all Finnish MRSA blood isolates (N=124), ii) 80 FIN-16 isolates, and iii) 112 FIN-4 isolates (excluding FIN-4 and FIN-16 blood isolates). For FIN-4 and FIN-16 MRSA isolate sets, the first isolate of every month between the years 1997 and 2006 (max. 12 isolates per year), if available, were chosen. For 2006, every eleventh isolate for each month was chosen (to avoid overlapping with the first ten isolates already included in the short-term analysis).

Pulsed-field gel electrophoresis

The 589 MRSA isolates were previously genotyped by pulsed-field gel electrophoresis (PFGE) according to the Harmony protocol as described previously

[11, 12]. The strain NCTC 8325 was used as a control. PFGE patterns were analysed by BioNumerics (Applied Maths, Kortrijk, Belgium) by using the Dice coefficient to analyse the similarity of the banding patterns, and the unweighted pair group method using arithmetic averages (UPGMA) for cluster analysis. As in previously published studies, a similarity cut-off of 80% [13] or the criterion of a difference of ≤ 6 bands [4] were used to assign a PFGE type. The PFGE types were considered different if a seven or more band difference occurred. PFGE subtypes were not assigned. PFGE profiles differing by more than six bands, compared to any other profile in the local PFGE database, were regarded as sporadic types.

Spa typing

The *spa* typing was done as described previously [5, 14]. Sequences were analyzed by the Ridom StaphType program (Ridom GmbH, Würzburg, Germany) [15]. Clustering of clonal complexes of related *spa* types was done by using the repeat pattern (BURP) algorithm of the Ridom StaphType software [16]. The default parameters of the software were used for the cluster definition: *spa* types were clustered if the calculated cost between members of a group was less than or equal to four and *spa* types shorter than five repeats were excluded. A bionehbourjoining tree of *spa* types was created by Splits Tree4 (version 4.10) program [17] by using cost values for analysing distances between *spa* types of blood isolates.

Multilocus sequence typing

Multilocus sequence typing (MLST) was performed as described previously [18]. Eight FIN-4 and 11 FIN-16 representative MRSA strains were selected for MLST, based on different *spa* typing results compared to the most common *spa* type of FIN-4

(t172) and FIN-16 (t067) strains. In addition, to study clonality of the typical FIN-16 MRSA strains with *spa* type t067, between 2002 and 2006, five strains were selected for MLST. Lineage assignment to clonal complexes (CCs) for each sequence type (ST) was performed by eBURST analysis using default stringent parameters [19].

SCC*mec* typing

Staphylococcal cassette chromosome *mec* (SCC*mec*) analysis was performed for the same 19 representative MRSA strains which went through the MLST analysis. The *ccr* type (*ccrAB1* to *ccrAB4*, or *ccrC*) and *mec* class (A, B, or C) within SCC*mec* were analyzed by PCR (M-PCR 1 and -2) as described previously by Kondo and co-workers [20].

Discriminatory power and Wallace's coefficient

The discriminatory power (discriminatory index, DI) of different genotyping methods were calculated using the Ridom StaphType software as described earlier [12, 21, 22]. The quantitative concordance between typing methods was analyzed by Wallace coefficient (W) [23, 24]. Wallace's coefficient value indicates the probability that two strains classified as the same type by one method are also classified as the same by another method. A high value of W coefficient indicates that the result obtained by a given typing method could have been predicted by the other method.

Cost and time analysis

The mean hands-on time and total time used for processing one set of isolates for *spa* typing (N=24) and PFGE (N=12) was estimated by stopwatch analysis of each step of the processes at least three times by three different persons. The time used for

151 analysing the results was included in the hands-on time. The material costs of both
152 methods were estimated based on material usage per isolate. Sequencing was
153 provided as a service from another laboratory.

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Results

MRSA blood isolates (years 1997-2006)

The 124 MRSA blood isolates belonged to 38 different *spa* types comprising eight *spa* clusters (*spa*-CC), including one without founder, and 10 singleton *spa* types (12 isolates). The most prevalent *spa* type was t067 (32%), and 33 *spa* types were found in less than four isolates of each (Table1). By PFGE, 25 different types were identified; six of these were sporadic.

In seven of eight *spa*-CCs, one PFGE type comprised the majority of isolates in each *spa*-CC (Table 1 and Fig. 1). For instance, among the 20 isolates of *spa*-CC2521/041, 18 were of PFGE type FIN-21 whereas one isolate was FIN-24 and another one was sporadic. Isolates showing other than the typical PFGE type of the particular *spa*-CC are indicated in Table 1. Isolates within *spa*-CC012 showed the most variable PFGE types (FIN-2, FIN-5, FIN-9, FIN-13, and two sporadic types). Seven of twelve isolates with singleton *spa* types had a distinct PFGE type (FIN-1, -12, -20, -14, and sporadic) from any other isolate and four of which (*spa* types t032, t148, t160, and t355) were also distant from any *spa*-CCs in the bionighbourjoining tree (Table 1 and Fig. 1).

Isolates of nine (FIN-4, -5, -7, -10, -11, -15, -16, -19, and -21) of the 25 PFGE types showed variation in *spa* types. (Table 1). Within isolates of four PFGE types (FIN-5, -10, -15, and -19), some *spa* types were not members of the same *spa*-CC, but were mostly singleton or excluded *spa* types. However, among all but one PFGE type (FIN-5), a single *spa* type dominated and the rest occurred only once. The three FIN-5 isolates showed each a different *spa* type. FIN-10 was a highly variable PFGE-type;

three isolates were t015, and five isolates of each showed a different *spa* type. As a whole 48% (59/124) of *spa* types of blood isolates were associated with multiple PFGE types or were recognised in Finland for the first time.

Among the blood isolates *spa* typing had higher discriminatory power than PFGE types as calculated by Ridom and PFGE had higher discriminatory power than the *spa*-CC (Table 2). *spa* type had a higher probability to predict PFGE types ($W=0.9009$) than PFGE types to predict *spa* types (0.7853) as calculated by Wallace coefficient. PFGE types performed better in predicting *spa*-CC ($W=0.9870$) than *spa*-CC in predicting PFGE types (0.7810).

MRSA FIN-4 and FIN-16 epidemic isolates

Of the 220 FIN-4 MRSA isolates between the years 1997 and 2006, nine *spa* types were found. The *spa* types belonged to one *spa* cluster, *spa*-CC 172, and to one singleton. The majority (87%, 191/220) of the isolates were of *spa* type t172. Three sequence types (ST) among eight uncommon *spa* types of FIN-4 isolates were identified by MLST: ST87 (N=1), ST59 (N=1) and ST375 (N=6) (Table 3.). All the STs belonged to one clonal complex (CC), CC59. Seven of eight isolates possessed SCC*mec* type IV and one isolate was non-typeable.

Twelve *spa* types were found among the 196 FIN-16 isolates between the years 1997 and 2006. All belonged to one *spa* cluster, *spa*-CC 002. The most prevalent *spa* type was t067 (88%). Among 11 *spa* types other than t067, three STs were found by MLST; ST5 (N=6), ST125 (N=3) and ST146 (N=2) (Table 3). The five FIN-16 isolates with *spa* type t067 were ST125. All the STs belonged to CC5. Among the

same 11 isolates, eight possessed SCC*mec* type IV, and the remaining three carried SCC*mec* type I, V, and a non-typeable SCC*mec* type. Because of excluded, singleton or nontypable *spa* type, 4% (8/220) of FIN-4 and 3% (5/196) of FIN-16 were not recognized as belonging to the same clonal lineage based on *spa* typing alone.

Sporadic isolates (year 2006)

Among the 49 sporadic isolates 29 *spa* types were found. The most prevalent *spa* type was t008 (12%). Sporadic isolates were divided into four *spa* clusters (*spa*-CC 002, *spa*-CC 172, *spa*-CC 008 and a cluster with no founder) and into 11 singleton *spa* types (14 isolates). Eighteen (18/49, 37%) of the sporadic isolates shared a *spa* type with the representatives of Finnish epidemic strains defined earlier by PFGE [12]. Forty-five (92 %) of the sporadic isolates associated with *spa* type which related to multiple PFGE types, previously unknown *spa* type among Finnish isolates, or an NT *spa* type result.

Cost and time analysis

Total times required to type 24 isolates by *spa* typing was 9 h 15 min, and 12 isolates by PFGE was 40 h. Hands-on times were 6 h 30 min for both methods. Approximate material cost for PFGE was 5 € per isolate and for *spa*-typing 9 € per isolates.

Discussion

Nationwide laboratory-based MRSA surveillance requires accurate typing with a possibility both to detect local outbreaks and to perform international comparisons. Ideally, the typing process should also be fast and economic. The aim of this study was to estimate the consequences of replacing PFGE with *spa* as a primary typing method in a centralized reference laboratory for the ability of local infection control bodies to perform surveillance and outbreak detection, which has been based on PFGE-determined strain nomenclature.

spa typing possessed a high probability to predict PFGE type, and was faster to perform, but more expensive than PFGE. The total time to perform PFGE was approximately four times longer than time required for *spa* typing. The hands-on times used for both methods were equal. However, some inaccuracy exists in estimating PFGE hands on time. The time required for interpretation of PFGE patterns was highly variable, and because of practical reasons, the number of isolates processed at a time was 12 in PFGE, whereas it was 24 in *spa* typing.

The 124 Finnish MRSA blood isolates from the period 1997 to 2006 were genetically diverse, comprising 38 *spa* types. According to a recent study of European invasive *S. aureus* covering the period 2006–2007, three of the most frequent *spa* types among MRSA isolates were t032, t008 and t041. *spa* type t041 was found to be common also in our study, comprising 13% of all blood isolates during the period 1997–2006 [25].

Although several *spa* types among blood isolates showed more than one PFGE type, in most instances the PFGE type was consistent within the *spa*-CC. Variation in

PFGE types occurred especially in *spa*-CC 12. Since PFGE typing had been performed earlier (between years 1997 and 2006), these results were not interpreted at the subtype level. This affected the discriminatory power of PFGE, and explains the discrepancy with several studies that have shown PFGE to be more discriminatory than *spa* typing [9, 14]. The two genotyping methods measure different markers of genetic variation in *S. aureus*. PFGE targets the whole genome whereas *spa* typing targets the polymorphic repeat region of the *spa* gene. The mutation rate of the given bacterial clone affects the pattern stability of PFGE [3, 26] and epidemiological interpretations of related PFGE patterns depend on the time and space scale of the study [26]. The *spa* region has been shown to be fairly stable [27, 28], although Kahl and co-workers demonstrated a rather high mutation rate for this region among *S. aureus* strains isolated from cystic fibrosis patients with persistent *S. aureus* infections [29]. However, the relation of the *spa* region variability to the overall evolution of the *S. aureus* genome is not yet fully understood, and may differ between *S. aureus* clones.

At the time of analysis, nearly half of the blood isolates had a *spa* type recognised in Finland for the first time, or associated with multiple PFGE types. Therefore, PFGE or another typing method is still required as has also been reported in other studies [7, 8]. Four sporadic isolates by PFGE would have been classified as non-unique by *spa* typing. However, among the two major MRSA strains (FIN-16 and FIN-21) among blood isolates, majority (90%) would have been consistently identified as t067 or t041, respectively, based on *spa* typing alone.

276 The FIN-4 isolates during 1997–2006 were largely homogenous (*spa*-CC172,
277 SCC*mec*IV) and the predominant *spa* type t172 was found throughout these years.
278 The FIN-4 isolates are known to be community-associated and found throughout
279 Finland. Between 2004 and 2006 FIN-4 comprised 10% of all MRSA isolates in
280 Finland [10]. *spa* types provided additional information in one instance where a small
281 cluster was recognised among the FIN-4 isolates with *spa* type t3627 (one extra repeat
282 compared to repeats in t172) in 2006. The strains were isolated from the same
283 healthcare centre during June and July. Three strains with the same *spa* type were also
284 found in 2002 and 2003 from the same healthcare district.

285

286 According to the content of the Ridom SpaServer, *spa* type t172 has been isolated in
287 eight European countries, but to the best of our knowledge, it does not belong to the
288 most prevalent type of strains in clinical settings outside the Nordic countries [19].
289 Recently, isolates with *spa* t172 were identified as the most commonly found MRSA
290 in municipal waste water in Sweden [30]. The genotypes recognized in waste water
291 corresponded to those in clinical settings, and therefore, likely originated from human
292 carriage.

293

294 The FIN-16 isolates between 1997 and 2006 were genetically close to each other, as
295 most had *spa* type t067, and all belonged to *spa*-CC002. However, variation on
296 SCC*mec* types was noticed among FIN-16 isolates, which can be a consequence of
297 different ancestors or the susceptibility of staphylococcal isolates to receive,
298 recombine, and/or replace different SCC*mec* elements [31]. During 1997–2000, the
299 *spa* type t002 was predominant among the FIN-16 isolates but it was replaced by *spa*
300 type t067 from 2001 onwards. Outbreaks of FIN-16 (t067) seem to be responsible for

the increase of reported MRSA cases observed from 1997 to 2004 [1] and between 2004 and 2006 FIN-16 comprised nearly one third of all MRSA isolates in Finland [32]. The same clone, MRSA-ST125-IV, has also been recognized as the most prevalent hospital-acquired-MRSA in Spain [8]. A clone closely related to FIN-16 (ST5, *spa* type t067 and SCCmec II) has also been indentified in Portuguese hospitals [33]. In addition, according to the content of the Ridom SpaServer, *spa* type t067 isolates have been identified in 13 different countries.

Using *spa* typing instead of PFGE would not have affected interpretation of the majority of the two most prevalent MRSA strains in Finland, FIN-4 and FIN-16. Nearly ninety percent of the *spa* types of FIN-4 and FIN-16 were consistent. For the rest, PFGE was additionally required because the isolate was non-typeable, the *spa* type (t002) obtained was known to be associated with multiple PFGE types, or it was previously unknown among Finnish isolates.

Using *spa* typing as the primary genotyping method will influence on the amount of isolates interpreted as sporadic types, since 37% defined as unique by PFGE shared a *spa* type with an epidemic strain. Most of the sporadic isolates would have required PFGE due to previously unknown *spa* type among Finnish isolates, an NT *spa* type result, or *spa* type associated with multiple PFGE types. However, the need for additional typing by PFGE will decrease when the knowledge on less common *spa* types and their associated PFGE types accumulates through adoption of the new typing scheme in Finland.

In our revised typing process, PFGE will follow *spa* typing if a *spa* type is recognised in Finland for the first time, a new *spa* type according to *spa* server is encountered, on occasions where a *spa* type is known to be associated with multiple PFGE types, or if the isolate is not typable by *spa* typing. In 2009, additional typing by PFGE was needed for 22% of isolates in Finland. *spa* typing qualifies as a first-line typing method, and can be adapted for laboratory-based MRSA surveillance in countries with a long history of band pattern –based strain nomenclature.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure caption

Fig 1 Bionehbourjoining tree of *spa* types of the blood isolates (N=124) excluding those (N=2) with less than five repeat units. Spa-CCs are shown by gray circles and the most prevalent PFGE-type of each spa-CC is indicated. S indicates a singleton *spa* type. Asterisk indicates the most prevalent *spa* type within spa-CC.

Table 1. MRSA blood isolates between 1997 and 2006 in Finland.

spa-CC^a	<i>spa</i> type	<i>spa</i> repeat	No. of isolates (N=124)	PFGE type	Isolation year/years
002	t002	26-23-17-34-17-20-17-12-17-16	2	FIN-3	2005, 2006
008	t008	11-19-12-21-17-34-24-34-22-25	3	FIN-7	2001, 2005, 2006
	t008	11-19-12-21-17-34-24-34-22-25	2	FIN-15	2001, 2004
	t008	11-19-12-21-17-34-24-34-22-25	1	FIN-25	2006
	t024	11-12-21-17-34-24-34-22-25	1	FIN-7	2005
	t068	11-19-19-12-21-17-34-24-34-22-25	2	FIN-7	2003, 2004
	t121	11-19-21-17-34-24-34-22-25	1	FIN-7	2006
	t596	11-19-12-21-17-34-34-24-34-22-25	1	FIN-7	2006
	t596	11-19-12-21-17-34-34-24-34-22-25	1	FIN-10	2002
012	t012	15-12-16-02-16-02-25-17-24-24	1	FIN-5	2006
	t018	15-12-16-02-16-02-25-17-24-24-24	1	FIN-5	1997
	t030	15-12-16-02-24-24	1	FIN-9	2005
	t037	15-12-16-02-25-17-24	4	FIN-2	1998, 1999, 2004
	t037	15-12-16-02-25-17-24	2	FIN-13	1998
	t037	15-12-16-02-25-17-24	2	sporadic	2002, 2004

015	t015	08-16-02-16-34-13-17-34-16-34	3	FIN-10	2005, 2006
	t583	08-16-02-16-34-13-16-34	1	sporadic	2002
	t630	08-16-02-16-34-17-34-16-34	1	FIN-10	2006
067	t067	26-23-17-34-17-20-17-12-17	39	FIN-16	2002-2006
	t067	26-23-17-34-17-20-17-12-17	1	sporadic	2002
	t1084	26-23-17-34-17-20-17-12	1	FIN-16	2005
	t3148	26-23-17-34-17-13-17-12-17	1	FIN-16	2005
	t442	35-17-34-17-20-17-12-17-16	1	FIN-16	2006
376	t044	07-23-12-34-34-33-34	2	FIN-11	1999, 2005
	t267	07-23-12-21-17-34-34-33-34	1	FIN-19	2006
	t376	07-23-12-34-34-34-33-34	1	FIN-11	2006
2521/041	t041	26-30-17-34-17-20-17-34-17-20-17-12-17-16	14	FIN-21	2004-2006
	t041	26-30-17-34-17-20-17-34-17-20-17-12-17-16	1	FIN-24	2005
	t041	26-30-17-34-17-20-17-34-17-20-17-12-17-16	1	sporadic	2004
	t1628	26-30-17-34-17-20-17-34-17-20-17-12-17-16-16	2	FIN-21	2006
	t2521	26-30-17-13-17-20-17-34-17-20-17-12-17-16	1	FIN-21	2004
	t3181	26-30-17-13-17-20-17-13-17-20-17-12-17-16	1	FIN-21	2004
no founder	t172	04-20-17-45-16-34	12	FIN-4	1999, 2001-2004, 2006
	t976	04-20-17-20-31-16-34	1	FIN-4	2005
singleton	t032	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	2	FIN-12	2000, 2006
	t127	07-23-21-16-34-33-13	2	FIN-19	2002, 2004
	t148	07-23-12-21-12-17-20-17-12-12-17	1	FIN-20	2005
	t160	07-23-21-24-33-22-17	1	FIN-14	1999
	t355	07-56-12-17-16-16-33-31-57-12	1	sporadic	2006
	t550	08-17-34-16-34	1	FIN-10	2005
	t562	11-19-21-12-21-17-34-22-25	1	FIN-1	2000
	t689	11-19-12-22-25	1	FIN-15	2004

	t2099	08-16-16-34-34	1	sporadic	2006
	t2163	01-12-16-16-16-02-16-02-25-24-24	1	FIN-5	2003
excluded	t1644	08-13-16-34	1	FIN-10	2004
	t3020	08-17-13-34	1	FIN-10	2004

^a*spa* types were clustered if the calculated cost between members of a group was less than or equal to four and *spa* types shorter than five repeats were excluded.

spa types marked in bold font showed multiple PFGE types.

Table 2. Discriminatory power of different genotyping methods among blood isolates.

Method	No. of different types	DI	95% CI
<i>spa</i>	38	0.866	[0.819-0.914]
spa-CC	17	0.811	[0.761-0.861]
PFGE	26	0.847	[0.798-0.895]

Table 3. *spa* typing, MLST and SCC*mec* results of FIN-4 and FIN-16 MRSA strains in Finland. *spa* types shorter than five repeats were excluded.

PFGE type	Year	No. of isolates studied ^a	<i>spa</i> type ^b (No. of isolates)	<i>spa</i> repeat	spa-CC	MLST ST	MLST CC	SCC <i>mec</i>
FIN-4	1997	8	t172	04-20-17-45-16-34	172			
	1998	11	t172	04-20-17-45-16-34	172			
	1999	12	t172	04-20-17-45-16-34	172			
	2000	12	t172	04-20-17-45-16-34	172			

	2001	12	t172	04-20-17-45-16-34	172			
	2002	12	t172 (9)	04-20-17-45-16-34	172			
			t216 (1)	04-20-17-20-17-31-16-34	172	87	59	IV
			t3627 (2)	04-20-17-24-45-16-34	172	375	59	IV
	2003	12	t172 (8)	04-20-17-45-16-34	172			
			t529 (1)	04-34	Excluded	375	59	IV
			t2380 (1)	04-02-17-45-16-34	172	375	59	IV
			t3627 (2)	04-20-17-24-45-16-34	172			
	2004	12	t172 (12)	04-20-17-45-16-34	172			
	2005	12	t172 (12)	04-20-17-45-16-34	172			
	2006	117	t172 (95)	04-20-17-45-16-34	172			
			t458 (1)	26	Excluded	375	59	IV
			t529 (2)	04-34	Excluded			
			t976 (1)	04-20-17-20-31-16-34	172	59	59	NT
			t2380 (3)	04-02-17-45-16-34	172			
			t3424 (2)	04-13	Excluded	375	59	IV
			t3627 (11)	04-20-17-24-45-16-34	172			
			t3718 (1)	04-12-45-16-34	Singleton	375	59	IV
			NT (1)	-	-			
FIN-16	1997	1	t002	26-23-17-34-17-20-17-12-17-16	002	5	5	IV
	1998	2	t002	26-23-17-34-17-20-17-12-17-16	002			
	1999	6	t002 (2)	26-23-17-34-17-20-17-12-17-16	002			
			t067 (1)	26-23-17-34-17-20-17-12-17	002			
			t214 (3)	26-23-17-34-17-20-17-12-17-16-16	002	5	5	IV
	2000	3	t002 (1)	26-23-17-34-17-20-17-12-17-16	002			
			t067 (1)	26-23-17-34-17-20-17-12-17	002			
			NT (1)	-	-			
	2001	8	t002 (2)	26-23-17-34-17-20-17-12-17-16	002			
			t067 (5)	26-23-17-34-17-20-17-12-17	002			
			t242 (1)	26-23-17-13-17-20-17-12-17-16	002	5	5	V
	2002	12	t067 (12)	26-23-17-34-17-20-17-12-17	002	125	5	

2003	12	t067 (11)	26-23-17-34-17-20-17-12-17	002	125	5	
		NT (1)	-	-			
2004	12	t067 (11)	26-23-17-34-17-20-17-12-17	002	125	5	
		t535 (1)	26-17-16	Excluded	5	5	NT
2005	12	t067 (12)	26-23-17-34-17-20-17-12-17	002	125	5	
2006	128	t067 (119)	26-23-17-34-17-20-17-12-17	002	125	5	
		t442 (3)	35-17-34-17-20-17-12-17-16	002	146	5	IV
		t548 (1)	26-23-17-34-17-20-17-12-16	002	146	5	IV
		t777 (1)	26-23-17	Excluded	125	5	IV
		t952 (1)	26-23-17-34-17-20-12-12-17-16	002	5	5	I
		t1094 (1)	23-17-34-17-20-17-12-17-16	002	5	5	IV
		t1991 (1)	08-17	Excluded	125	5	IV
		t3109 (1)	26-23-17-34-17-17-12-17	002	125	5	IV

^a 1997-2005: the first FIN-4 and FIN-16 strain of each month, if available.

2006: every eleventh FIN-4 and FIN-16 strain of each month, if available.

^bNT, non-typeable