



Abstract: P529

SeqNet.org: a European-wide certification trial for sequence-based typing of microbial pathogens

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and the SeqNet.org participants

Objectives: SeqNet.org is an initiative of currently 28 laboratories from 20 European countries in order to establish a European network of excellence for sequence based typing of microbial pathogens. The principle goal of SeqNet.org is to establish unambiguous, electronic portable, easily comparable typing data for local infection control and national and European surveillance of sentinel microorganisms. Here, we describe (i) the harmonization of sequencing methods for sequence based typing, (ii) the capacity building for DNA sequencing in diagnostic microbiology, and (iii) the certification trail for sequence-based typing of MRSA.

Methods: After the 'kick-off' meeting in Münster, Germany (November 2004), the participants received a protocol for typing of MRSA by *Staphylococcus aureus* protein A gene (*spa*) typing. Subsequent, five strains, 5 DNAs, and 5 forward and reverse chromatogram files of representative and well characterized MRSA strains were distributed to all participating laboratories to be typed until the end of 2005. The typing results were analysed and synchronized with the central server by using the Ridom StaphType software.

Results: All participating European laboratories built up capacities for sequence-based typing and established the *spa* typing method for typing of MRSA in the laboratories. Until today, the typing results for the certification trial were submitted by 24 of the participating laboratories. Each laboratory determined 2,783 bp (range, 206-422 bp per strain) and all participants reported exactly the same *spa* type for each of the analysed isolates and for the additional 5 chromatograms. Therefore, the intra- and inter-laboratory reproducibility of the sequencing results was 100% each. Online synchronization of the results proved the rapid exchange of high quality typing data based on nucleotide sequencing.

Conclusion: The SeqNet.org initiative enables laboratories European-wide to build up capacity for sequence-based methods. The *spa* typing results proved the unambiguous and highly reproducible nature and high portability of sequence data. The usage of a standardized nomenclature based on the software enabled an easy exchange of data.

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www.SeqNet.org

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Summary

SeqNet.org is an initiative of currently 35 laboratories from 22 European countries in order to establish a European network of excellence for sequence based typing of microbial pathogens (1). The principle goal of SeqNet.org is to establish unambiguous, electronic portable, easily comparable typing data for local infection control and national and European surveillance of sentinel micro-organisms. Here, we describe (i) the harmonization of sequencing methods for sequence based typing, (ii) the capacity building for DNA sequencing in diagnostic microbiology, and (iii) the certification trail for sequence-based typing of MRSA.

Objectives of SeqNet.org

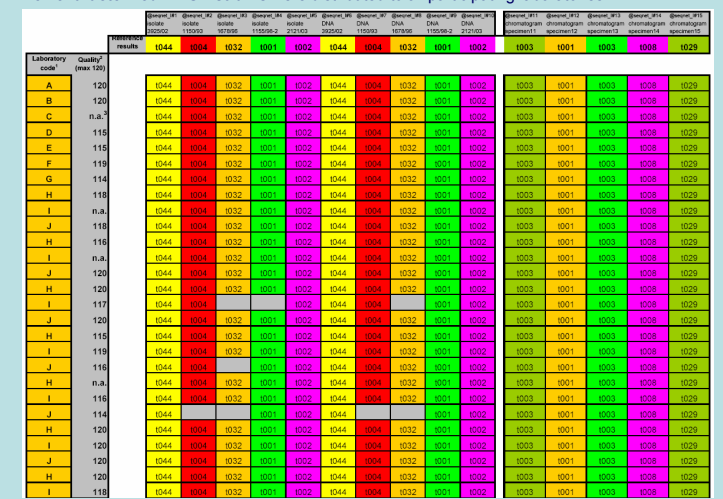
- Harmonization of sequencing methods for sequence based typing of *Staphylococcus aureus* and capacity building for DNA sequencing**
S. aureus is the most common cause of nosocomial infections/outbreaks. MRSA is of nosocomial and public health concern in many European countries. As rapid, unambiguous, and affordable typing method *spa* typing is used (Figure 1). Up to now, 4 *spa* typing workshops were held in Münster (2x), Ulm, Prague. One workshop in 11/2005 was a joint initiative of SeqNet and EARSS and funded EARSS.
- QC/QA for DNA sequencing (external quality control scheme)**
A first feasibility study showed 100% intra- and inter-laboratory comparability between 11 international laboratories (2).
Certification: 30 SeqNet.org laboratories took place in the trial. Five strains, 5 DNAs, and 5 (fir) chromatogram files of well characterized MRSA strains. Objectives: - Capacity building for sequence technology
- High quality sequence based typing
- Inter-lab comparability of typing data
Time of trial: 12 months
- Improving the access to sequence based microbial typing results and the transfer of data at international level**
Networking of the participants by the Ridom StaphType™ software, which allows a common nomenclature and quantitative measure of the sequence quality as described before (3)
- Disseminating the project results**

Table 1. SeqNet.org participants

Organization	Contact	City	Country
1 Institut für Hygiene, Mikrobiologie und Tropenmedizin	H Mittermayer	Linz	Austria
2 Österreichische Agentur für Gesundheit und Ernährungssicherheit	F. Allerberger	Vienna	Austria
3 Hôpital Erasme - Centre for Molecular Diagnostic (CMD)	M Struelens	Brussels	Belgium
4 National Center of Infectious and Parasitic Diseases	T. Kantardziev	Sofia	Bulgaria
5 National Institute of Public Health	H Zemlickova	Prague	Czech Republic
6 Laboratory of Veterinary Medicine (LABVET)	O. Meler	Prague	Czech Republic
7 Hvidovre Hospital (advisory board)	H Westh	Hvidovre	Denmark
8 Statens Seruminstitut	R. Skov	Copenhagen	Denmark
9 National Public Health Institute	J. Vuopio-Varkila	Helsinki	Finland
10 Hôpital Edouard Bellet	J. Blain	Lyon	France
11 Institute of Hygiene of the University of Münster	A.W. Friedrich (coordinator)	Münster	Germany
12 Institute of Medical Microbiology of the University Hospital Münster	K. Becker, B. Kahl	Münster	Germany
13 University of Würzburg	U. Vogel	Würzburg	Germany
14 Robert Koch-Institut	W. Witte (coordinator)	Wernigerode	Germany
15 University of Applied Sciences/Public Health	R. Reintjes	Hamburg	Germany
16 University of Athens	N. Lekakis	Athens	Greece
17 "Johann Béla" National Center for Epidemiology	M. Fuzsi	Budapest	Hungary
18 Istituto Superiore di Sanità	A. Paritosi	Rome	Italy
19 P. Stradins Clinical University Hospital	E. Miklaševič	Riga	Latvia
20 University of Malta	A. McEnattin	Malta	Malta
21 Laboratorium Microbiologie Twente Achterhoek	R. Hendrix	Enschede	The Netherlands
22 National Institute of Public Health and the Environment	X. Huisdens	Bilthoven	The Netherlands
23 University Hospital Maastricht	E. Stobberingh J. Scheres (advisory board)	Maastricht	The Netherlands
24 Akershus University Hospital	O. Bjalkheim	Lørenskog	Norway
25 Telesko	Y. Tsvetan	Skiel	Norway
26 National Institute of Public Health	W. Hryniewicz (advisory board)	Warsaw	Poland
27 Instituto de Tecnologia Química e Biológica (ITQB)	H. de Lencastre (advisory board)	Oeiras	Portugal
28 National Institute for Research and Development for Microbiology and Immunology	I. Cofite	Bucharest	Romania
29 Scottish MRSA Reference Laboratory	D. Morrison	Glasgow	Scotland (UK)
30 University of Ljubljana, Medical Faculty, Institute of Microbiology and Immunology	M. Mueller-Trommsdorff	Ljubljana	Slovenia
31 Lund University Hospital	A.-C. Petersson	Lund	Sweden
32 Swedish Institute for Infectious Disease Control (Smittskyddsinstitutet)	S. Hesselmar	Solna	Sweden
33 Sahlgrenska University Hospital	C. Weiland-Olsson	Göteborg	Sweden
34 Universitätsspital Basel	R. Frei	Basel	Switzerland
35 Health Protection Agency	A. Kearns	London	UK

Results of the certification trial

Sequence typing of 5 strains, 5 DNAs (same as strains), and 5 forward and reverse chromatograms of well characterized MRSA strains were distributed to all participating laboratories.



* anonymized laboratory code, not in the order of the participants; * overall quality of sequences performed by laboratories; * results sent via email, synchronization with *spa* server missing

- > 27 of 30 (90%) laboratories completed in time the certification trial
- > Each laboratory determined 2,783 bp (range, 206 to 422 bp per strain)
- > 397 of 405 (98%) of *spa* typings were completed and correct
- > Results were synchronized with the central *spa* server
- > 100% of the *spa* typing data synchronized was correct
- > Overall quantitative quality of *spa* sequences was 117 of 120 (excellent)

Conclusions

- > All participating European laboratories built up capacities for sequence-based typing and established the *spa* typing method for typing of *S. aureus* in their laboratories.
- > More than 12.000 isolates has been *spa* typed yet and synchronized via the *spa* Server
- > Certification trial showed high reproducibility and inter-lab comparability of *spa* typing data
- > Only data of excellent quality was synchronized. Low quality of data was not synchronized
- > Capacity building of sequenced based typing (e.g. via hands-on workshops) must be intensified

References

(1) Friedrich A. W., Witte W., Hryniewicz W., de Lencastre H., Hryniewicz W., Scheres J., Westh H. 2005. SeqNet.org - a European laboratory network for sequence based typing of microbial pathogens. *Communicable Disease and Public Health* 8: 100-101. Available from: www.cdc.gov/dpdx

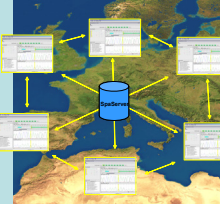
(2) Aronoff-Spencer M., Bays P., de Lencastre H., Dupont A., Gough M.C., Gilman J., Friedrich A., Harman D., Holmes A., Hryniewicz W.K., Kearns A.M., Malmgren A., Malmgren A., Rasmussen J.K., Spangberg E., Strommenger B., Struelens M.J., Towner F.C., Thomas J., Vogel D., Westh H., Wu Z., Witte W. 2005. High inter-laboratory reproducibility of DNA sequence-based typing of *Staphylococcus aureus* in a European network. *PLoS ONE* 4: e12624.

(3) Hryniewicz W., Chen W., Hryniewicz A., Chmura M., Tomaszek M., Witte W. 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using net-server software for the rapid determination and database management. *J. Clin. Microbiol.* 41: 5441-5445.

(4) Frey P.M., Borchert A.C., Schumacher M., von Lencastre H., Hryniewicz W.K., Westh H., de Lencastre H., Witte W. 2005. Molecular typing of methicillin-resistant *Staphylococcus aureus* by using net-server software for the rapid determination and database management. *J. Clin. Microbiol.* 43: 1834-1840.

spaServer

(A) Synchronization process within SeqNet.org is obtained by using the Ridom StaphType software (2)



(B) Typing data on *spa* Server developed by Ridom and curated by SeqNet.org (25.3.2006)

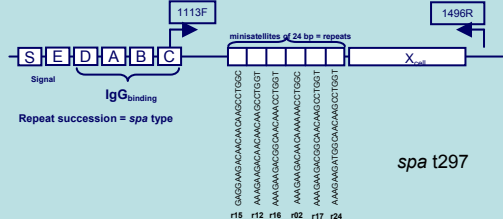
39 countries (22 active in SeqNet.org)
91 laboratories (35 active in SeqNet.org)

1.303 *spa* types
107 *spa* repeats
12.904 *S. aureus* isolates with database input (92% MRSA)

> A European network has been established synchronizing all *spa* typing data through a central *spa* server developed by Ridom and curated by SeqNet.org in order to obtain

> a unique nomenclature with high quality data is available for more than 1300 *spa* types.

Figure 1. Genetic organization of the *S. aureus* protein A gene (*spa*), modified from Fréney et al. (4) and the following Ridom repeat nomenclature (4) used within SeqNet.org



115 112 116 117 112 124