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International circumpolar surveillance interlaboratory quality control program for *emm* typing of *Streptococcus pyogenes*, 2011–2015

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Abstract

In 2011, an interlaboratory quality control (QC) program for *emm* typing group A streptococci (GAS) was incorporated into existing international circumpolar surveillance QC programs. From 2011 – 2015, 35 GAS isolates were distributed to three laboratories; *emm* type-level concordance was 100%, while the overall sub-type level concordance was 83%.

Keywords

Quality control; *emm* typing; *Streptococcus pyogenes*

The International Circumpolar Surveillance (ICS) program was established to enhance the surveillance of infectious diseases of special interest to the circumpolar regions (Parkinson et al., 1999; Zulz et al., 2009). This program was initiated in 1999 and initially focused on the surveillance of invasive pneumococcal disease (IPD) in the U.S. Arctic (Alaska) and northern Canada. Soon thereafter, other northern countries joined (Greenland, Iceland, Norway, Finland, and Sweden) and the program has expanded to cover surveillance of other invasive bacterial diseases including *Neisseria meningitidis, Haemophilus influenzae, Streptococcus pyogenes* (Group A *Streptococcus*, GAS) and *S. agalactiae* (Group B Streptococcus, GBS) (Parkinson et al., 2008).

Essential to the success of any international surveillance program is the need to be able to compare results between testing laboratories, which are often generated by using different methodologies. In 1999, with the formation of the ICS program for the surveillance of IPD, an interlaboratory quality control (QC) program was introduced serving as an external proficiency testing mechanism for the serotyping and antibiotic susceptibility testing of *Streptococcus pneumoniae* (Reasonover et al., 2011). A similar program that monitors laboratory proficiencies in the serogrouping of *N. meningitidis* and serotyping of *H. influenzae* was introduced into the ICS program in 2005 (Tsang et al., 2012). In 2011, a

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program that monitors laboratory proficiency in the *emm* typing of GAS was co-developed by the Centers for Disease Control and Prevention's Arctic Investigation Program located in Anchorage, Alaska and the Public Hsealth Agency of Canada's National Microbiology Laboratory located in Winnipeg, Ontario. In addition to the Anchorage and Winnipeg laboratories, the Provincial Laboratory for Public Health, located in Edmonton, Alberta also participates in this program. Here we describe the interlaboratory quality control program for *emm* typing of GAS isolates and present data collected from the first 5 years of this program (2011–2015).

From 2011 through 2015, five panels consisting of seven isolates of *S. pyogenes* were distributed annually among the three participating laboratories. Each participating laboratory was responsible for the selection and distribution according to a pre-defined distribution schedule. GAS isolates were selected to represent a variety of *emm* sequence types excluding isolates known to be non-typeable. The selected isolates were labeled according to the year and distribution event, e.g. 15GAS01, 15GAS02, etc. and testing personnel were not involved in isolate selection. All isolates were placed in charcoal transport media or on chocolate agar slants and shipped according to International Air Transportation Association regulations.

DNA lysates for *emm* typing were prepared as follows: Anchorage and Edmonton prepared DNA lysates according to the lysate preparation protocol described on the Centers for Disease Control and Prevention (CDC) website (http://www.cdc.gov/streplab/protocol-emm-type.html); Winnipeg prepared DNA lysates using the Epicentre Bacterial Quick Extract kit (Mandel Scientific, Guelph, Ontario, Canada). The *emm* gene was amplified using the primer sequences listed on the CDC website. PCR master mix and cycling conditions varied between labs (Table 1). Purification of PCR products to serve as the sequencing templates were prepared as follows: ExoSAP-IT® (Affymetrix, Santa Clara, CA; Anchorage), PCRClean DX™ (Aline Biosciences, Woburn MA; Winnipeg) and QIAquick PCR purification kit for gel blocks (Qiagen, Germantown, MD; Edmonton). All three laboratories used the CDC protocol for sequencing the *emm* gene. Sequence analysis was performed by a BLAST search on the CDC streptococcal *emm* sequence database (http://www2a.cdc.gov/ncidod/biotech/strepblast.asp) to designate *emm* sequence type.

A standardized report form to ensure consistent data collection was sent by the distributing laboratory with each QC panel. The information collected on the report form included isolate identifiers and *emm* results to the type level, (e.g. *emm* 4.4was reported as *emm* 4). The rationale for reporting results to the type-level only were two-fold: 1) historically, reference laboratories routinely reported *emm* results only to the type level, therefore this EAQ program was similarly structured; and 2) *emm* type-level data correlates more closely to the traditional M serotypes so type level results were readily recognizable to what clients were used to receiving. The distributing laboratory is responsible for compiling a summary report of the results, and if discrepant results are noted, the distributing lab may initiate a discussion to assist with troubleshooting.

During the first five years (2011–2015) of this quality control program, a total of 35GAS isolates were distributed among the three participating laboratories. The *emm* types of the

QC organisms are described in Table 2. The distributed isolates represented 24 *emm* types of which *emm* types 2, 3, 4, 6, 8, 34, 41, 75, 76, 77, 78, 82, 87, 94, 95, 108, and 118 were represented once while *emm* types 1, 12, 11, 28, 53, 89 and 91 were each represented two or more times. Over the five year period of this QC program our results showed 100% concordance in GAS *emm* typing at the type-level among the participating laboratories. However, one laboratory had differences at the subtype level, e.g. 1.38 instead of 1.0, 94.2 instead of 94.0 (Table 2) leading to an overall subtype-level concordance of 83%. These differences could be due to sequencing errors or the quality of the sequences obtained.

Strain characterization of GAS was traditionally based on serological identification of the M protein which is a major surface protein and an important GAS virulence factor. Classical serologic M typing inmost laboratories has been replaced by *emm* typing, which in almost all cases, predicts the classical M serotype (Athey et al., 2014; Beall et al., 1991) and to date over 200 *emm*-types have been recognized. Although greater than 200 *emm* types have been described, only a limited number of types have been associated with invasive GAS disease. For example, in Alaska between 2001 and 2013, 516 cases of invasive GAS disease were reported of which 422 had isolates available for laboratory analysis. Among these 422 isolates, 51 *emm* types were identified (Rudolph et al., 2016).

Other phenotypic techniques used to characterize GAS isolates include the characterization of cell surface T protein antigens and serum opacity factor production (Johnson et al., 2006). While both of these methods can add an additional level of resolution when combined with *emm* typing, the availability of antisera is limited. Many laboratories have therefore moved away from using phenotypic techniques and rely strictly on molecular methods for GAS typing.

Because infectious diseases are a global threat, their prevention and control is aided by international collaboration of which surveillance is a key component. The International Circumpolar Surveillance program creates an infectious disease surveillance network of hospital and public health laboratories and authorities throughout Arctic regions. This surveillance program allows for the standardized collection, comparison and sharing of laboratory and epidemiologic data on invasive GAS disease and assists in the formulation of prevention and control strategies. Another international surveillance collaboration includes the strep-EURO project launched in 2002 to enhance understanding of the epidemiology of invasive GAS disease in Europe (Neal et al., 2007). One of the objectives of this project was to improve GAS strain characterization by harmonizing methods and establishing an external quality assurance program (EQA). The EQA study included both phenotypic and molecular typing methods. Among 15 participating centers that performed *emm* typing, an overall concordance of 98% for *emm* type-level data was achieved, similar to what is reported in this study. Also, there was variation in the interpretation of *emm* subtypes, which was also reported here and highlights difficulties when assigning subtypes. High concordance (90%) for *emm* typing was also reported in a previous EQA exercise conducted from 1997–1999 by this same group (Efstratiou et al., 2000). The few incorrect results reported in this exercise were mainly due to mismatches and variability in the interpretation of sequence data due to the lack of an officially recognized and designated database which has since been established. It should be noted that the aforementioned EQA exercises were

conducted prior to the release of new parameters for assigning types to *emm* sequence data. New types are now identified on the basis of sharing less than 92% sequence identity over the first 90 bases encoding the deduced processed M protein of the *emm* type reference strain. This report represents the first EQA study performed since the changes took effect and highlights the accurate and reliable nature of this GAS typing method.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Table 1

Methods and reagents used for *emm* typing of *Streptococcus pyogenes*.

Anchorage		**Winnipeg**		**Edmonton**	
Reagents	**per Reaction**	**Reagents**[a]	**per Reaction**	**Reagents**	**per Reaction**
AccuPrime™ Super MixII[b]	10.0 µL	5× buffer	7.0 µL	10× buffer + $MgCl_2$	4.0 µL
Primer 1 (70 pM/µL)[c]	1.0 µL	5 mM $MgCl_2$	3.2 µL	dNTPs (2.5 mM)	4.0 µL
Primer 2 (70 pM/µL)[c]	1.0 µL	dNTPs (5 mM)	1.4 µL	Primer 1 (10 µM)	5.6 µL
dH_2O	6.0 µL	Primer 1 (100 µM)	0.5 µL	Primer 2 (10 µM)	5.6 µL
Template	2.0 µL	Primer 2 (100 µM)	0.5 µL	HotStar Taq[d] (5 U/µL)	0.6 µL
Total	20 µL	GoTaq® (5 U/µL)	0.2 µL	dH_2O	18.2 µL
		dH_2O	20.2 µL	Template	2.0 µL
		Template	2.0 µL	Total	40 µL
		Total	35 µL		
Cycling conditions		Cycling conditions		Cycling conditions	
94 °C 1 min 1 cycle		94 °C 5 min 1 cycle		94 °C 15 min 1 cycle	
94 °C 15 sec 10 cycles		94 °C 1 min 40 cycles		94 °C 15 sec 10 cycles	
46.5 °C 30 sec 10 cycles		47.5 °C 1 min 40 cycles		46.5 °C 30 sec 10 cycles	
72 °C 1 min 10 cycles		72 °C 2 min 40 cycles		72 °C 1 min 10 cycles	
94 °C 15 sec 20 cycles		72 °C 7 min 1 cycle		94 °C 15 sec 20 cycles	
46.5 °C 30 sec 20 cycles		4 °C Hold		46.5 °C 30 sec 20 cycles	
72 °C 1 min 15 sec with a 10 sec increment for each subsequent 19 cycles 10 cycles				72 °C 1 min 15 sec with a 10 sec increment for each subsequent 19 cycles	
				10 cycles	
72 °C 10 min 1 cycle				72 °C 10 min 1 cycle	
4 °C Hold				4 °C Hold	

[a] PCR Master Mix (Go Taq® Flexi PCR Kit, Promega, Madison, WI).

[b] Life Technologies, Grand Island, NY.

[c] PCR primers from http://www.cdc.gov/streplab/protocol-emm-type.html.

[d] HotStar Taq DNA Polymerase (Qiagen, Redwood City, CA).

Table 2

Emm type results for the quality control isolates of the International Circumpolar Surveillance program, 2011–2015.

Year	No. of isolates	*emm* types	% Concordance to type level (no. of concordant results/total no. of tests)
2011	7	1.0*, 4.0, 12.8, 28.0, 75.0, 87.0, 89.0	100 (7/7)
2012	7	3.1, 76.1, 78.3, 82.0*, 89.0(2), 94.0*	100 (7/7)
2013	7	2.0, 6.0, 11.0, 41.2, 53.0*, 77.0, 91.0	100 (7/7)
2014	7	1.0, 8.0, 12.0*, 28.0*, 89.0, 91.0, 118.0	100 (7/7)
2015	7	1.0, 11.0, 34.0, 53.1, 91.0, 95.0, 108.1	100 (7/7)

*Denotes differences at the subtype level for these *emm* types (1.38, 94.2, 82.3, 53.1, 12.76, 28.4).