

# Emerging Invasive Group A *Streptococcus* M1<sub>UK</sub> Lineage Detected by Allele-Specific PCR, England, 2020

## Appendix

**Appendix Table 1.** Evaluation of *rofA* and *pstB* allele-specific primers for lineage assignment by using genome-sequenced *emm1* strains from 2017–2018\*

Year	Lineage†	Total no. strains	No. strains yielding PCR products with allele-specific primer pairs‡			
			<i>rofA</i> , M1 <sub>global</sub>	<i>rofA</i> , M1 <sub>UK</sub>	<i>pstB</i> , M1 <sub>global</sub>	<i>pstB</i> , M1 <sub>UK</sub>
2017	M1 <sub>global</sub>	3	3	0	3	0
	M1 <sub>UK</sub>	6	0	6	0	6
2018	M1 <sub>global</sub>	5	5	0	5	0
	M1 <sub>UK</sub>	13	0	13	0	13

\*Allele-specific PCR results performed on extracted DNA were consistent with whole-genome sequencing of DNA from 27 *emm1* noninvasive *S. pyogenes* isolates (n = 9 from 2017, n = 18 from 2018, enriched for M1<sub>global</sub> strains). Sequences for *emm1* test strains were submitted to the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>) under project no. PRJEB58440.

†Lineage was assigned on the basis of whole-genome sequencing results.

‡Primer pairs for *rofA* and *pstB* genes that detect M1<sub>global</sub> and M1<sub>UK</sub> lineages are listed in the Table (main text).

**Appendix Table 2.** Accession numbers of genome-sequenced strains used to validate allele-specific PCR used to detect all sublineages\*

M1 lineage	Year	Accession no.	Sample ID
M1 <sub>13snps</sub>	2013	ERS1594714	PHEGAS005
M1 <sub>13snps</sub>	2013	ERS1594852	PHEGAS127
M1 <sub>13snps</sub>	2015	ERR1733723	GASEMM2799
M1 <sub>13snps</sub>	2015	ERR1734520	GASEMM2970
M1 <sub>23snps</sub>	2013	ERS1594734	PHEGAS025
M1 <sub>23snps</sub>	2013	ERS1594744	PHEGAS035
M1 <sub>23snps</sub>	2013	ERS1594757	PHEGAS048
M1 <sub>23snps</sub>	2013	ERS1594864	PHEGAS137
M1 <sub>global</sub>	2013	ERS1594798	PHEGAS168
M1 <sub>global</sub>	2013	ERS1594822	PHEGAS097
M1 <sub>global</sub>	2014	ERR1732733	GASEMM1027
M1 <sub>global</sub>	2015	ERR1734897	GASEMM2755
M1 <sub>UK</sub>	2013	ERS1594722	PHEGAS013
M1 <sub>UK</sub>	2014	ERR1733140	GASEMM0629
M1 <sub>UK</sub>	2015	ERR1733678	GASEMM3027
M1 <sub>UK</sub>	2016	ERS1594947	PHEGAS285

\*Sequences from European Nucleotide Archive (<https://www.ebi.ac.uk/ena>) were previously listed in reference (4) (main text).

**Appendix Table 3** Validation of *rofA*, *pstB*, and *gldA* allele-specific primers for lineage assignment by using genome-sequenced *emm1* strains, including intermediate sublineages\*

Lineage†	Total no. strains	No. strains yielding PCR products with allele-specific primer pairs‡					
		<i>rofA</i> , M1 <sub>global</sub>	<i>rofA</i> , M1 <sub>UK</sub>	<i>gldA</i> , M1 <sub>global</sub>	<i>gldA</i> , M1 <sub>UK</sub>	<i>pstB</i> , M1 <sub>global</sub>	<i>pstB</i> , M1 <sub>UK</sub>
M1 <sub>global</sub>	4	4	0	4	0	4	0
M1 <sub>13snps</sub>	4	0	4	4	0	4	0
M1 <sub>23snps</sub>	4	0	4	0	4	4	0
M1 <sub>UK</sub>	4	0	4	0	4	0	4

\*Whole-genome sequenced *S. pyogenes emm1* strains from 2013 and 2014 (n = 16, accession nos. in Appendix Table 2) were analyzed by using *rofA*, *gldA*, and *pstB* allele-specific PCR primers. Strains were selected for the presence of 0/27, 13/27, 23/27, or 27/27 single nucleotide polymorphisms specific for M1<sub>UK</sub> (4 isolates of each). Allele-specific PCR results were consistent with whole-genome sequencing.

†Lineage was assigned on the basis of whole-genome sequencing results.

‡Primer pairs for *rofA*, *gldA*, and *pstB* genes that detect M1<sub>global</sub> and M1<sub>UK</sub> lineages are listed in the Table (main text).

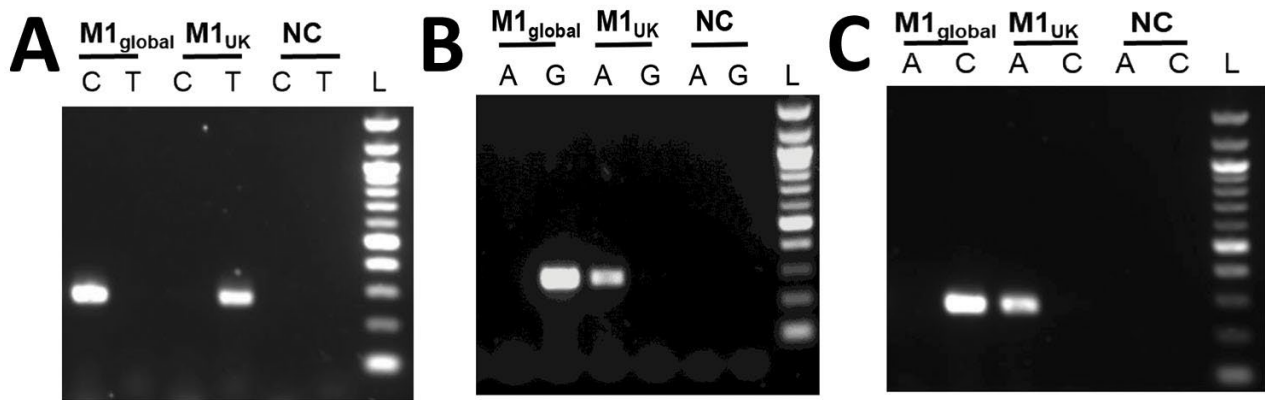
**Appendix Table 4.** Use of allele-specific PCR to test 305 invasive *emm1* *Streptococcus pyogenes* isolates of unknown lineage that were collected in 2020\*

Lineage†	No. strains yielding PCR products with allele-specific primer pairs‡						Total
	<i>rofA</i> , M1 <sub>global</sub>	<i>rofA</i> , M1 <sub>UK</sub>	<i>gldA</i> , M1 <sub>global</sub>	<i>gldA</i> , M1 <sub>UK</sub>	<i>pstB</i> , M1 <sub>global</sub>	<i>pstB</i> , M1 <sub>UK</sub>	
M1 <sub>global</sub>	27	0	27	0	27	0	27
M1 <sub>UK</sub>	0	278	0	278	0	278	278

\*All invasive *emm1* strains (n = 305) submitted to the reference laboratory for *emm* genotyping and identified as *emm1* were evaluated by allele-specific PCR. A total of 278 isolates yielded PCR products consistent with the M1<sub>UK</sub> lineage. No intermediate isolates were identified.

†Lineage was inferred by results of allele-specific PCR.

‡Primer pairs for *rofA*, *gldA*, and *pstB* genes that detect M1<sub>global</sub> and M1<sub>UK</sub> lineages are listed in the Table (main text).



**Appendix Figure.** Gel electrophoresis demonstrating allele-specific PCR to distinguish between M1<sub>global</sub> and M1<sub>UK</sub> *Streptococcus pyogenes* lineages. PCR products after amplification of DNA from M1<sub>global</sub> and M1<sub>UK</sub> strains of *S. pyogenes* are shown. (A) *rofA* SNP primers; C and T denote nucleotide differences used in *rofA* forward primers. (B) *gldA* SNP primers; A and G denote nucleotide differences used in *gldA* A or G forward primers. (C) *pstB* SNP primers; A and C denote nucleotides used in *pstB* A or C forward primers. Primer pairs for *rofA*, *gldA*, and *pstB* genes that detect M1<sub>global</sub> and M1<sub>UK</sub> lineages are listed in the Table (main text). DNA was isolated from control strains BHS0151 (M1<sub>global</sub>) and BHS581 (M1<sub>UK</sub>) that have been previously described (3, main text) and then sequenced. Genome sequences are in the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>) under accession nos. ERS1020136 (M1<sub>global</sub>) and ERS1020603 (M1<sub>UK</sub>). L, 100 bp DNA ladder; NC, negative control.