**Supporting information**

# Evasion of killing by human antibody and complement through multiple variations in the surface oligosaccharide of *Haemophilus influenzae*

Sarah E. Clark, Department of Microbiology, University of Pennsylvania, Philadelphia, PA, USA; Kara R. Eichelberger, Department of Microbiology, University of Pennsylvania, Philadelphia, PA, USA; Jeffrey N. Weiser*, Department of Microbiology, University of Pennsylvania, PA, USA

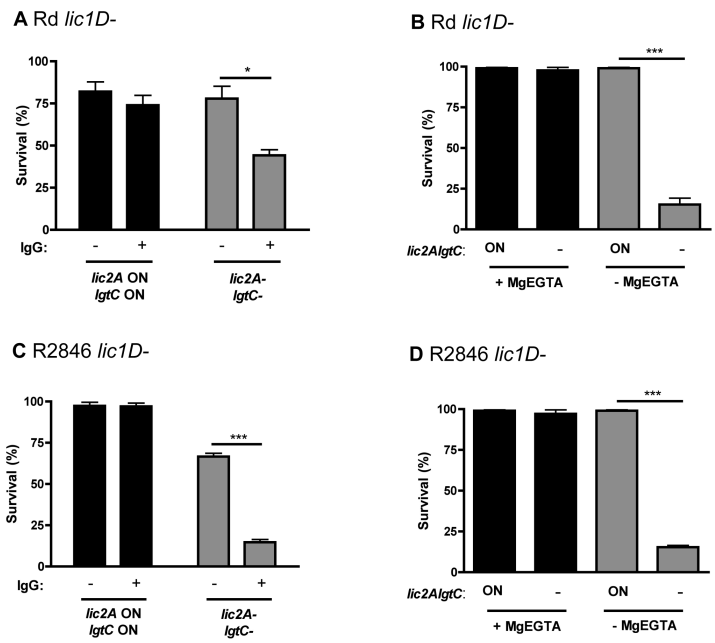
* Corresponding Author

**Supplementary Table 1.** Primers used for the construction of *H. influenzae* mutants

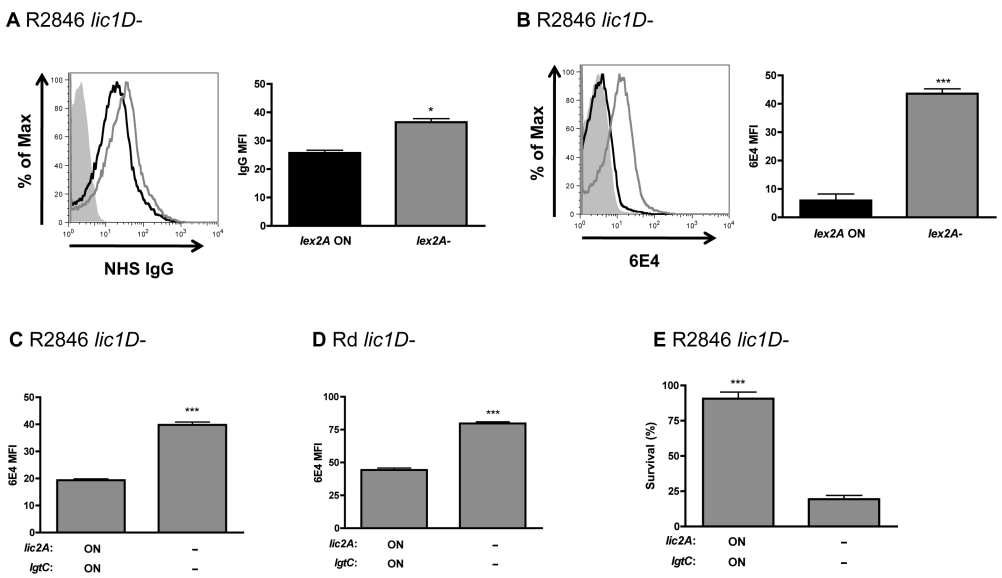
| Target gene | Direction | Sequence | Reference |
|---|---|---|---|
| *lex2A* amplify | F | 5'-ACCCCTTTGCATTCAACCGCT-3' | This study |
| | R | 5'-CCGAATGGTTCTGCGGAGGGC-3' | |
| *lex2A* XmaI | F | 5'-GCGGCGGGGCCCGCTTGCTTGCCAAGACTATC-3' | This study |
| | R | 5'-GCGGCGGGGCCCGCAAGCAAGCAAAGAGTGAC-3' | |
| *aad9* | F | 5'-GCGGCGGGGCCCATCGATTTTCGTTCGTGAATACATGTT-3' | This study |
| | R | 5'-GCGGCGGGGCCCTATGCAAGGGTTTATTGTTTTCTAAAATCTGA-3' | |
| *siaP* | F | 5'-GTTCACACAGGAGCGAAT-3' | (Severi *et al.*, 2005) |
| | R | 5'-TACAGAGTATGCTGCTGC-3' | |

## References

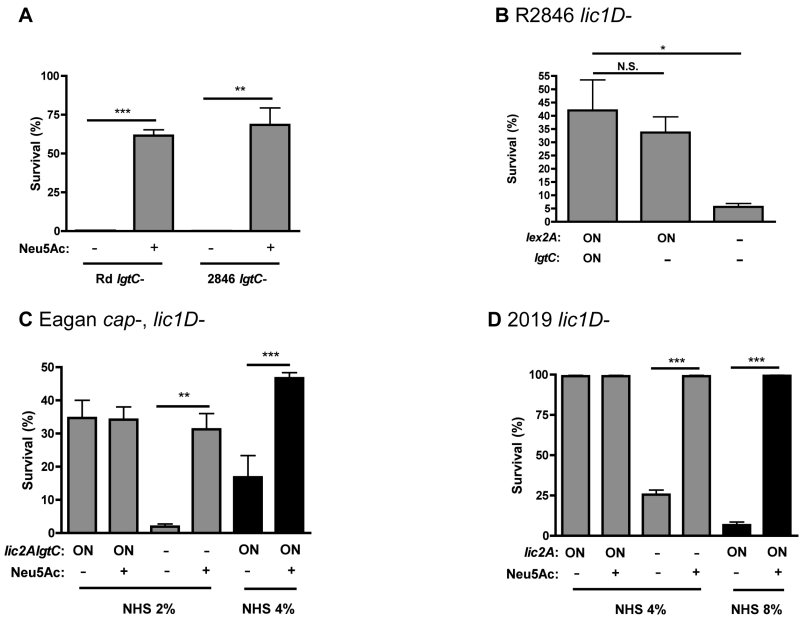
Severi, E., G. Randle, P. Kivlin, K. Whitfield, R. Young, R. Moxon, D. Kelly, D. Hood & G. H. Thomas, (2005) Sialic acid transport in *Haemophilus influenzae* is essential for lipopolysaccharide sialylation and serum resistance and is dependent on a novel tripartite ATP-independent periplasmic transporter. *Mol Microbiol* **58**: 1173-1185.




**Supplementary Figure 1.** Di-galactoside expression protects against classical pathway complement-mediated killing**.** Bactericidal assays in baby rabbit serum (BRS) with or without purified IgG for *lic2A* and *lgtC* phase-on variants and *lic2A*- mutants in Rd *lic1D*- (A, 4% BRS). Bactericidal assays in human serum with or without Mg-EGTA treatment for *lic2A* and *lgtC* phase-on variants and *lic2A*- mutants in Rd *lic1D*- (B, 2% normal human serum, NHS). Bactericidal assays in BRS with or without purified IgG in R2846 *lic1D*- (C, 15% BRS). Bactericidal assays in human serum with or without Mg-EGTA-treatment in R2846 *lic1D*- (D, 4% NHS). Data shown are means and SEM. Statistical analysis ($n \geq 3$) was performed by an unpaired *t*-test; $^{*}P<0.05$, $^{***}P<0.001$.




**Supplementary Figure 2.** Di-galactoside expressing variants have dual resistance to human IgG binding and mAb 6E4 binding and bactericidal activity**.** Histogram and graphical summary of human IgG (A) and mAb 6E4 (B) binding to *lex2A* phase-on variants (black) compared to a *lex2A*- mutant (grey) in R2846 *lic1D*-, with graphical mean fluorescence intensity (MFI) summaries. Summary of MFI for mAb 6E4 binding to *lic2A* and *lgtC* phase-on variants isolated following passage in normal human serum (NHS) compared to *lic2A*- mutants in R2846 *lic1D*- (C) and Rd *lic1D*- (D). Bactericidal assays in human serum for *lic2A* and *lgtC* phase-on variants isolated following passage in mAb 6E4 and baby rabbit serum (BRS) compared to *lic2A*- mutants in R2846 *lic1D*- (E, 4% NHS). Data shown are means and SEM. Statistical analysis ($n \geq 3$) was performed by an unpaired *t*-test; $^{*}P<0.05$, $^{***}P<0.001$.




**Supplementary Figure 3.** Additive and independent effects on bacterial survival in human serum for LPS modifications in multiple *H. influenzae* strains**.** Bactericidal assays in human serum for a Rd *lic2A* phase-on, *lgtC*- mutant (2% normal human serum, NHS) and a R2846 *lic2A* phase-on, *lgtC*- mutant (4% NHS) with or without sialic acid (Neu5Ac) added (+) to growth medium (A). Also, bactericidal assays in human serum for *lex2A* and *lgtC* phase-on variants compared to a *lex2A* phase-on, *lgtC*- mutant and a *lex2A-, lgtC*- mutant in R2846 *lic1D*- (B, 4% NHS). Individual and collective contributions of *lic2A*, *lgtC* and sialic acid (Neu5Ac) modifications in Eagan *cap*-, *lic1D*- (C) and 2019 *lic1D*- (D). Bactericidal assays were performed at the concentration of normal human serum (NHS) indicated. Data shown are means and SEM. Statistical analysis ($n \geq 3$) was performed by an unpaired *t*-test; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.