

## Fluorescent and electrochemical detection of nuclease activity associated with *Streptococcus pneumoniae* using specific oligonucleotide probes

Garazi Goikoetxea, Khadija-Tul Kubra Akhtar, Alona Prysiashniuk, Baris A. Borsa, Mehmet Ersoy Aldag, Murat Kavruk, Veli C. Ozalp, Frank J. Hernandez\*

\* Corresponding author: Frank J. Hernandez, Email: [frank.hernandez@liu.se](mailto:frank.hernandez@liu.se)

### Supplementary Information

**Table S1.** Oligonucleotide sequences used in this study.

| Oligo | Name               | Sequence (5' to 3')                                 |
|-------|--------------------|---|
| 1     | DNA                | 5'-FAM-TCT-CGT-ACG-TTC-TQ2-3'                       |
| 2     | DNA-Poly A         | 5'-FAM-AAA-AAA-AAA-AAA-TQ2-3'                       |
| 3     | DNA-Poly T         | 5'-FAM-TTT-TTT-TTT-TTT-TQ2-3'                       |
| 4     | DNA-Poly C         | 5'-FAM-CCC-CCC-CCC-CCC-TQ2-3'                       |
| 5     | DNA-Poly G         | 5'-FAM-mUmUmU-GGG GGG GGG-TQ2-3'                    |
| 6     | RNA                | 5'-FAM-ucu-cgu-acg-uuc-TQ2-3'                       |
| 7     | RNA-Poly A         | 5'-FAM-aaa-aaa-aaa-aaa-TQ2-3'                       |
| 8     | RNA-Poly U         | 5'-FAM-uuu-uuu-uuu-uuu-TQ2-3'                       |
| 9     | RNA-Poly C         | 5'-FAM-ccc-ccc-ccc-ccc-TQ2-3'                       |
| 10    | RNA-Poly G         | 5'-FAM-mumumu-ggg-ggg-ggg-TQ2-3'                    |
| 11    | All 2'O-Methyl     | 5'-FAM-mUmCmU-mCmGmU-mAmCmG-mUmUmC-TQ2-3'           |
| 12    | RNA-Pyr-2'O-Methyl | 5'-FAM-mumcmu-mcgmu-amcg-mumumc-TQ2-3'              |
| 13    | RNA-Pur-2'O-Methyl | 5'-FAM-macu-mgmgu-macmg-mguc-TQ2-3'                 |
| 14    | All 2'Fluoro       | 5'-FAM-fUfCfU-fCfGfU-fAfCfG-fUfUfC-TQ2-3'           |
| 15    | RNA-Pyr-2'Fluoro   | 5'-FAM-fUfCfU-fCgfU-afCg-fUfUfC-TQ2-3'              |
| 16    | RNA-Pur-2'Fluoro   | 5'-FAM-ucu cfGu fAcfG uuc-TQ2-3'                    |
| 17    | P1 probe           | 5'-FAM- mUmUmUmU-fUfA-mUmUmUmU- BMN-Q1 -3'          |
| 18    | P2 probe           | 5'-FAM- mUmUmUmU-fUfAfU-mUmUmUmU- BMN-Q1 -3'        |
| 19    | P3 probe           | 5'-FAM- mUmUmUmU-fUfAfAfU-mUmUmUmU BMN-Q1 -3'       |
| 20    | Fc-P3 probe        | 5'-Ferrocen- mUmUmUmU-fUfAfAfU-mUmUmUmU - Thiol -3' |

#### Key:

A, C, T, G = DNA

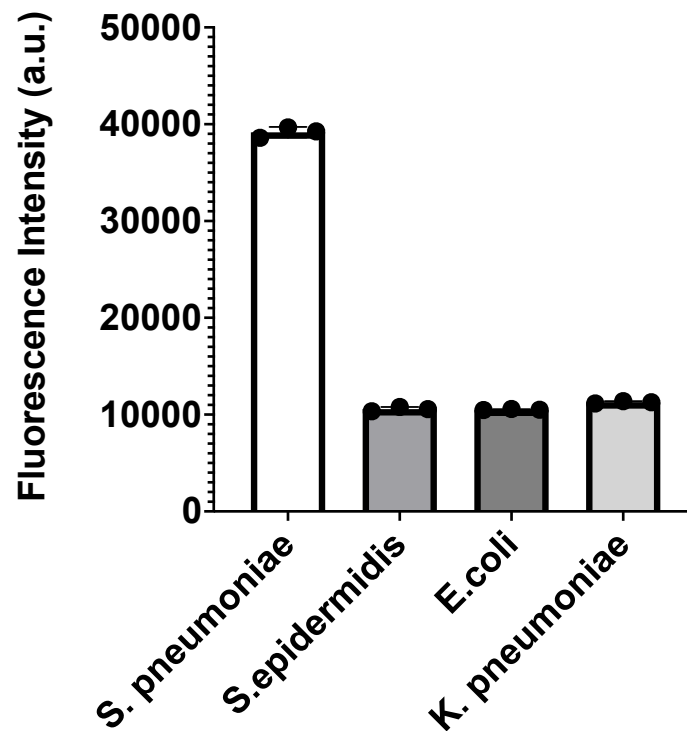
A, c, u, g = RNA

mA, mC, mU, mG = 2'O-Methyl modification

fA, fC, fU, fG = 2'Fluoro modification

**Table S2.** Verification of clinical samples

| Isolate                  | Sample origin         | Verification method |
|--------------------------|-----------------------|---------------------|
| <i>S. pneumoniae</i> CS1 | Sputum                | VITEK 2             |
| <i>S. pneumoniae</i> CS2 | Endotracheal aspirate | VITEK 2             |
| <i>S. pyogenes</i> CS1   | Throat                | VITEK 2             |
| <i>S. pyogenes</i> CS2   | Wound                 | VITEK 2             |
| <i>S. agalactiae</i> CS1 | Blood                 | VITEK 2             |
| <i>S. agalactiae</i> CS2 | Urine                 | VITEK 2             |



**Figure S1.** Specificity studies involving other human pathogens. The specific detection of *S. pneumoniae* was assessed using three relevant bacterial controls. All experiments were conducted in triplicate (indicated by dots), and the standard deviation was represented as the error.