

Supplemental Data

Table S1: *Haemophilus influenzae* 3TEC-PCR wild-type assay specificity panel

Organism	Strain	3TEC-PCR Result
Inclusivity Panel		
<i>H. influenzae</i> reference strains		
<i>H. influenzae</i> (b, Type Strain)	DSM 4690	+
<i>H. influenzae</i> (a)	NCTC 8465	+
<i>H. influenzae</i> (b)	DSM 10001	+
<i>H. influenzae</i> (b)	DSM 11969	+
<i>H. influenzae</i> (b)	DSM 11970	+
<i>H. influenzae</i> (b)	DSM 24049	+
<i>H. influenzae</i> (c)	NCTC 8469	+
<i>H. influenzae</i> (d)	DSM 11121	+
<i>H. influenzae</i> (e)	NCTC 8472	+
<i>H. influenzae</i> (f)	DSM 10000	+
Exclusivity Panel		
<i>Haemophilus</i> reference strains (excl. <i>H. influenzae</i>) with <i>N. meningitidis</i> and <i>S. pneumoniae</i> type strains		
<i>H. actinomycescomitans</i>	DSM 8324	-
<i>H. actinomycescomitans</i>	DSM 11122	-
<i>H. aphrophilus</i>	NCTC 11096	-
<i>H. avium</i>	DSM 18557	-
<i>H. ducreyi</i>	DSM 8925	-
<i>H. ducreyi</i>	NCTC 11479	-
<i>H. equigenitalis</i>	DSM 10668	-
<i>H. felis</i>	DSM 21192	-
<i>H. haemoglobinophilus</i>	DSM 21241	-
<i>H. haemolyticus</i>	CCUG 12834	-
<i>H. haemolyticus</i>	CCUG 15312	-
<i>H. haemolyticus</i>	CCUG 15642	-
<i>H. haemolyticus</i>	CCUG 24149	-
<i>H. haemolyticus</i>	CCUG 34110	-
<i>H. haemolyticus</i>	CCUG 36015	-
<i>H. haemolyticus</i>	CCUG 36016	-
<i>H. haemolyticus</i>	CDC-M19501	-
<i>H. haemolyticus</i>	CDC-M21127	-
<i>H. haemolyticus</i>	CDC-M21621	-
<i>H. haemolyticus</i>	NCTC 10839	-
<i>H. paracuniculus</i>	DSM 21452	-
<i>H. paragallinarum</i>	DSM 18554	-
<i>H. parahaemolyticus</i>	DSM 21417	-
<i>H. parainfluenzae</i>	DSM 8978	-
<i>H. paraphrohaemolyticus</i>	DSM 21451	-
<i>H. parasuis</i>	DSM 21448	-
<i>H. pittmaniae</i>	DSM 17240	-
<i>H. pittmaniae</i>	DSM 21203	-
<i>H. segnis</i>	NCTC 10977	-
<i>H. somnus</i>	CCUG 12839	-
<i>N. meningitidis</i> (type strain)	NCTC 10025	-
<i>S. pneumoniae</i> (type strain)	DSM 20566	-

DSM, Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures; NCTC, National Collection of Type Cultures; CCUG, Culture Collection, University of Gothenburg, Sweden; CDC, Centre for Disease Control; +, positive; -, negative.

Table S2: Synthetic DNA gBlocks Gene Fragment templates

SNP0 (without SNP):

500 bp sectional copy of the *H. influenzae* *fucK* gene diagnostic target

GTTTGCTACTCGTGGAGAAATTTATGTCAGCGCATTACAACATATGGCAAATAAACTCAAAA
ACGGTTTATCCGTGCTACATCAAGTCAGTCAGTTCCAAGCTAAAAGCTTAATTTGTGTTGGG
GGTGGTTCAAAAAATGTATTGTGGAATCAAATCCGTGCAAACACCTTAAATTTACCGATTGA
TGTTGTGGACATTTCTGAAAGTACTGTACTTGGTGCCGCAATGTTTACCTTTGCAGGCGTAGG
CATTTACGAAAATGTAAACGCGGCACAGCAAGCGATGCAACCAACAAGAAAACGAATTTA
CCCAAAGTAGAAGGAACAACAAAATGCTAAAAGGTATTCACCCAGCTCTTTCTCCTGAATT
ACTAAAAACCTCGCCGAAATGGGGCATGGCGATGAGATAGTTCTAGCTGACGCCCATTTT
CCCGCACATTCACCTACATAAAAAATGTAATACGTGCAGATGGTATATCTATAGATATTTTACT
TGAAGCA

SNP1 (with SNP):

SNP0 sequence with an SNP in the target region of the *H. influenzae* wild-type forward primer/probe

GTTTGCTACTCGTGGAGAAATTTATGTCAGCGCATTACAACATATGGCAAATAAACTCAAAA
ACGGTTTATCCGTGCTACATCAAGTCAGTCAGTTCCAAGCTAAAAGCTTAATTTGTGTTGGG
GGTGGTTCAAAAAATGTATTGTGGAATCAAATCCGTGCAAACACCTTAAATTTACCGATTGA
TGTTGTGGACATTTCTGAAAGTACTGTACTTGGTGCCGCAATGTTTACCTTTGCAGGCGTAGG
CATTTACGAAAATGTAAACGCGGCACAGCAAGCGATGCAACCAACAAGAAAACGAATTTA
CCCAAAGTAGAAGGAACAACAAAATGCTAAAAGGTATTCACCCAGCTCTTTCTCCTGAATT
ACTAAAAACCTCGCCGAAATGGGGCATGGCGATGAGATAGTTCTAGCTGACGCCCATTTT
CCCGCACATTCACCTACATAAAAAATGTAATACGTGCAGATGGTATATCTATAGATATTTTACT
TGAAGCA

Bold and underline, SNP.

Table S3: *H. influenzae* 3TEC-PCR wild-type assay limit of detection (LOD) probit analysis

Genome copy concentration	Replicates tested / Replicates detected
32	12 / 12
16	12 / 12
8	12 / 12
4	12 / 11
2	12 / 10
1	12 / 7
LOD with 95% probability: 4.1 genome copies per reaction	