

Specific primers for PCR detection

1. AFF-I-2 primer sequences:

AFF-I-2-F: 5'-CAGTCAGGAAAGTGGGAAAGC-3'

AFF-I-2-R: 5'-AGGTCTGTATAGTGCCGTCTG-3'

2. Stx2-2 primer sequences:

Stx2-2-F: 5'-GACAACGGACAGCAGTTATACC-3'

Stx2-2-R: 5'-CCACTCTGACACCATCCTCTC-3'

PCR reaction system:

10 X PCR buffer (25mM Mg ²⁺)	3 µl
Forward primer (5µM)	0.1 µl
Reverse primer (5µM)	0.1 µl
dNTPs(20mM)	0.1 µl
Taq DNA polymerase (5U / µl)	0.2 µl
Template DNA	2 µl
dH ₂ O	24.5 µl
Total volume	30µl

PCR conditions:

95° C	5min	
95° C	30sec	} 30 cycles
55-62° C	30sec	
72° C	30sec	
72° C	5min	

Sizes of PCR products:

358 bp (AAF_I-2)

338 bp (stx2-2)

Amplicons should be analyzed on a 1.5%-3% agarose gel, stained with EB and the result should be recorded. Only both positives for the two pairs of primers are considered the possible new outbreak strains in Germany this year.