# CNIEL Study

## The Effectiveness of Detection of Highly Pathogenic Shiga Toxin Producing *Escherichia coli* (STEC-HP) using Commercial Kits

Dairy companies routinely detect pathogenic STEC on milk and dairy products in their laboratories (or in third-party reference laboratories) using commercial kits. In France, testing is performed to detect the simultaneous presence of virulence genes (*stx* and *eae*) and the five major STEC serotypes (O157:H7, O26:H11, O145:H28, O103:H2 and O111:H8) of concern.

Three dairy associations in France, ANICAP, CNIEL, and General Confederation of Sheep Milk Producers and Industrialists, conducted a study using 4 commercial kits for the detection of STEC-HP in dairy matrices (milks and cheeses). The 4 kits analyzed were:

1. Assurance® GDS *MPX TOP 7* STEC, by Merck (formerly BioControl GDS)
2. GeneDisc® Plate STEC TOP 7, by Pall
3. BAX® System Real-Time PCR Assay – STEC Screening (*stx* and *eae*), from Hygiena
4. GENE-UP® EHEC, bioMérieux

Tests were performed using 6 different dairy matrices: raw cow's milk, raw goat's milk, raw sheep's milk, raw milk cow's cheese (pressed dough type), raw milk goat's cheese (lactic curd) and raw milk sheep's cheese (blue cheese type). The cheeses were inoculated with STEC-HP strains at a young stage.

Kit performance was evaluated based on 3 major criteria:

1. Practicality of the kits – including kit component storage, time to results, and similarities to the reference method
2. Relative level of detection – compared to the reference method
3. Relative sensitivity – compared to the reference method (ISO/TS 13136:2012)

As these kits are considered "alternative" methods, it is vital that they produce equivalent or better results than the reference (ISO) method. This means they must be user-friendly, provide a comparable (or improved) level of detection and sensitivity, and give results in a shorter time frame (to justify using the kit rather than the reference method).

# Results

1. **Practicality**
    a. All kits had similar storage criteria.
    b. 3 kits provided confirmation of positive results on Day 2 of testing: BAX, GeneDisc, and GENE-UP.
    c. Note: The reference method and 2 kits (BAX, Assurance) do not have a discordance procedure. GENE-UP and GeneDisc did. (A discordance procedure is used when results don't align; it is an additional test to resolve the discrepancy in results from a known sample and the test performed) .
2. **Level of Detection (LOD 50%)**
    a. The ISO 16140-2:2016 value of <2.5 for RLOD was used as a passing criterion.
    b. 3 kits satisfied the RLOD <2.5 limit for serotype O103.H2: BAX, GENE-UP, and GeneDisc.
    c. Only 2 kits satisfied the RLOD limit for serotype O26:H11: BAX and GENE-UP.
    d. Note: Results were dependent on the matrix tested but BAX outperformed all other kits on all matrices tested because the BAX assay has been designed for and validated on multiple matrices. The BAX System technology is designed for detecting low levels of contamination.
3. **Relative Sensitivity**
    a. A relative sensitivity of 53.70% to 91.07% was observed in the alternative methods (depending on matrix tested) versus 80.36% to 84.91% for the control method, and the relative accuracy of the alternative methods ranged from 71.90% to 88.43%
    b. Three kits (BAX, GENE-UP and GeneDisc) met the relative sensitivity requirements (table 4 of ISO 16140-2:2016) for the combined matrices.

# Conclusions

1. The BAX® System Real-Time PCR Assay – STEC Screening (*stx* and *eae*) had the highest relative accuracy with values from 85% to 95% and the smallest deviation value (-2, ND-PD)(ND = negative deviation, PD = positive deviation).
2. The BAX system showed the best LOD 50% values for all the matrices tested.
3. This third-party data demonstrates the advantages of the BAX system over other competitor kits – better sensitivity, higher accuracy, and a lower level of detection. Customers can feel confident that low levels of contamination are detected with few, if any, false-positive or false-negative results.