### Preparation of BEC28 VLPs

Total RNA was extracted with QIAamp Viral RNA Mini Kit (Qiagen) from 170 μL of intestinal content sample from an Italian calf infected with BEC strain BEC/28IT identified in a survey performed in Northern Italy. The poly(A)-containing RNA was subjected to reverse transcription using the oligo(dT)20 and SuperScript® III First-Strand Synthesis System (Invitrogen), following the manufacturer's instructions. Amplification of the entire capsid region by PCR was performed by a first PCR with primers J11U (5'-CCATCAACCATTGGATTTTGAC (1) and FwVP (5´- ataagaatg cgg ccg cgt aaa tga aga tga ctg ac-3'), followed by a second PCR, semi-nested, with primers FwVP and RORF2 (5'-Cggggt accgaattcagaagccatcaag-3') containing *Not*I and *Kpn*I, respectively. The DNA fragment obtained, flanked by restriction enzyme sites *Not*I and *Kpn*I, was ligated into the transfer vector, pFastBac™ 1 (Invitrogen) digested with the same enzymes. The bacmid with the entire BEC ORF2 (called BACBEC28, Acc # GQ397857) was transfected into the Sf9 insect cell line and the high titre BACBEC28 virus stock solution obtained was used to purify assembled VLPs, as confirmed by EM visualization. Sf9 cells were infected with high titre BACBEC28 and incubated at 27°C. Seven days postinfection, when a diffuse cytophatic effect was observable, cells and supernatant were collected. The VLPs were purified from cell culture supernatant and cell lysate by ultracentrifugation through a 30% sucrose cushion (wt/vol), followed by CsCl density gradient (1.362 g/cm$^3$) at 30.000 rpm for 24 hours at 12° C.

1. **Smiley, J. R., A. E. Hoet, M. Traven, H. Tsunemitsu, and L. J. Saif.** 2003. Reverse transcription-PCR assays for detection of bovine enteric caliciviruses (BEC) and analysis of the genetic relationship among BEC and human caliciviruses. J. Clin. Microbiol. 42:5214-5224.

**Figure A1:** Dendrogram, drawn using Bionumerics software packages (Applied Maths, Belgium) method UPGMA, was based on the entire ORF2. GenBank accession no., origin, and genotype are reported for all strains. Strains involved in this study are indicated in boldface type.

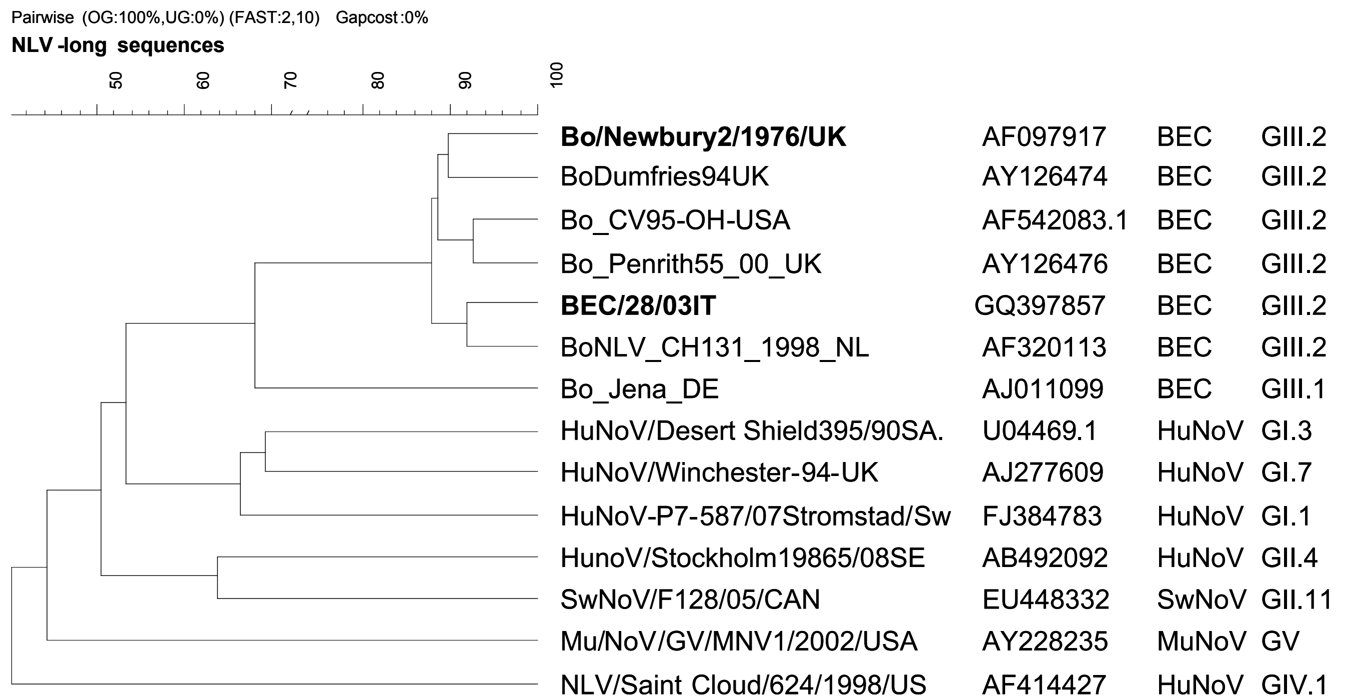

Table A1. Neoglycoconjugates used to determine the carbohydrate specificity of BEC28 VLPs

| **Trivial name** | **OLIGOSACCHARIDE STRUCTURE**[A] |
|---|---|
| Tn | GalNAcα-R1 |
| α−galactose monosaccharide | Galα-R1 |
| α−fucose monosaccharide | Fucα-R1 |
| A disaccharide | GalNAcα3Galβ-R1 |
| B disaccharide | Galα3Galβ-R1 |
| | Galα2Galβ-R1 |
| | Galα6Glcβ-R1 |
| Core 5 | GalNAcα3GalNAcα-R1 |
| H disaccharide | Fucα2Galβ-R1 |
| Forsmann disaccharide | GalNAcα3GalNAcβ-R1 |
| Core 8 | Galα3GalNAcα-R1 |
| Type 2 precursor | Galβ4GlcNAcβ-R1 |
| Tαβ | Galα3GalNAcβ-R1 |
| A trisaccharide | GalNAcα3(Fucα2)Galβ-R1, R2 |
| B trisaccharide | Galα3(Fucα2)Galβ-R1, R2 |
| H type 1 | Fucα2Galβ3GlcNAcβ-R1 |
| H type 2 | Fucα2Galβ4GlcNAcβ-R1, R2 |
| H type 3 | Fucα2Galβ3GalNAcα-R1 |
| αGal trisaccharide | Galα3Galβ4GlcNAcβ-R1 |
| Gb3 (Pk) | Galα4Galβ4Glcβ-R1 |
| iGb3 | Galα3Galβ4Glcβ-R1 |

| | |
|---|---|
| P1 trisaccharide | Galα4Galβ4GlcNAcβ-R1 |
| Lewis a | Galβ3(Fucα4)GlcNAcβ-R1 |
| Lewis x | Galβ4(Fucα3)GlcNAcβ-R1 |
| 3'-Sulfo-Lewis a | Su-O-3Galβ3(Fucα4)GlcNAcβ-R1 |
| 3'-Sulfo-Lewis x | Su-O-3Galβ4(Fucα3)GlcNAcβ-R1 |
| A type 2 | GalNAcα3(Fucα2)Galβ4GlcNAcβ-R1 |
| B type 2 | Galα3(Fucα2)Galβ4GlcNAcβ-R1 |
| Lewis b | Fucα2Galβ3(Fucα4)GlcNAcβ-R1, R3 |
| Lewis y | Fucα2Galβ4(Fucα3)GlcNAcβ-R1, R2 |
| Sialyl-Lewis a | NeuAcα2,3Galβ3(Fucα4)GlcNAcβ-R1, R3 |
| Sialyl-Lewis x | NeuAcα2,3Galβ3(Fucα4)GlcNAcβ-R1, R3 |
| 6-sulfo Sialyl-Lewis x | NeuAcα2,3Galβ3(Fucα4)(Su-O-6)GlcNAcβ-R1 |
| Tk | GlcNAcβ3(GlcNAcβ6)GlcNAcβ3Galβ-R1 |
| Lacto-N-tetraose (LNT) | Galβ3GlcNAcβ3Galβ4Glcβ-R3 |
| Lacto-N-neotetraose (LNnT) | Galβ4GlcNAcβ3Galβ4Glcβ-R3 |
| α-Gal-Lewis x | Galα3Galβ4(Fucα3)GlcNAcβ-R1 |
| α-Gal pentasaccharide | Galα3Galβ4GlcNAcβGalβ4Glcβ-R1 |
| Sialyl-Lewis x pentasaccharide | NeuAcα2,3Galβ3(Fucα4)GlcNAcβ3Galβ-R1 |
| Sialyl-lacto-N-neotetraose (Sia-LNnT) | NeuAcα2,3Galβ4GlcNAcβ3Galβ4Glcβ-R1 |
| Lactoneofucopentaose I (LNF I) | Fucα2Galβ3GlcNAcβ3Galβ4Glcβ-R1, R3 |
| Lacto-N-fucopentaose II (LNF II) | Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ-R3 |
| Lacto-N-fucopentaose III (LNF III) | Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ-R3 |
| A hexasaccharide | GalNAcα3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ-R3 |
| A heptasaccharide | GalNAcα3(Fucα2)Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ-R3 |

[a]Oligosaccharides were used coupled to either polyacrylamide via an 3 carbon spacer (R1), or to human serum albumin via either a p-aminophenylethyl spacer (R2) or an acetyl phenylenediamine spacer (R3).