Table 1: Bio-markers developed for the diagnosis and study of bacterial virulence genes.

Bio-marcadores	Target gene description	Primers sequence (5´- 3´)	Product (pb)	Amplificação
	Gene for diagnosis			
1	*aaiC* [a,c] – ilha ativadora do gene *aggR*, *E. coli* enteroagregativa (EAEC)	S: ATTGTCCTCAGGCATTTCAC AS: ACGACACCCCTGATAAACAA	215	20'' a 95ºC, 20'' a 57ºC, 1' a 72ºC
2	*aggR* [b,c] – regulador de aderência agregativa, EAEC	S: ATGAAATTAAAACAAAATATCGA AS: TCATTGGCTTTTAAAATAAGTCAA	798	30'' a 95ºC, 30'' a 58ºC, 1' a 72ºC
3	*hipO* [a,c] – hipurato hidrolase de *C. jejuni*	S: ATGATGGCTTCTTCGGATAG AS: GCTCCTATGCTTACAACTGC	176	30'' a 95ºC, 30'' a 51ºC, 45" a 72ºC
4	*ask* [a,c] – aspartato quinase de *C. coli*	S: GGTATGATTTCTACAAGCGAG AS: ATAAAAGACTATCGTCGCGTG	502	
5	glyA [a,c] – serina hidroximetiltransferase de *C. lari*	S: TAGAGAGATAGCAAAAGAGA AS: TACACATAATAATCCCACCC	251	30'' a 95ºC, 30'' a 46ºC, 45" a 72ºC
	Virulence gene			
6	*pet* [b,c] – toxina codificada por plasmídio, EAEC	S: TGACAGTGGATCAGGCGTGT AS: TTCTGTGCGCCAAGAATGAC	558	
7	*pic* [a,d] – proteína envolvida em colonização, EAEC	S: TTCAGCCGAAAGACGAAATCG AS: TCTGCGCATTCATACCAACAT	518	30'' a 95ºC, 30'' a 55ºC, 1' a 72ºC
8	*astA* [b,d] – toxina termo-estável agregativa, EAST1, EAEC	S: GCCATCAACACAGTATATCCGA	112	30'' a 95ºC, 30'' a 55ºC, 1' a 72ºC
9	*aap* [b,d] – proteína anti-agregativa, dispersina, EAEC	AS: CGATATTATTTAACCCATTCGG	356	

Continuation of Table 1: Bio-markers developed for the diagnosis and study of bacterial virulence genes.

Biomarkers	Target gene description	Primers sequence (5´- 3´)	Product (pb)	Amplificação
10	*cdtA* [a,d] – toxina citoletal distensora (CDT) porção, *C. jejuni*	S: TTGGCGATGCTAGAGTTTGG AS: ACCGCTGTATTGCTCATAGGG	175	30'' a 95ºC, 30'' a 56ºC, 45" a 72ºC
11	*cdtB* [a,d] – CDT porção B, *C. jejuni*	S: CTCGCGTTGATGTAGGAGCTA AS: GCAGCTAAAAGCGGTGGAGTA	418	
12	*cdtC* [a,d] – CDT porção C, *C. jejuni*	S: AGCCTTTGCAACTCCTACTGG AS: GCTCCAAAGGTTCCATCTTC	270	
13	flaA [a,d] – flagelina A, *C. jejuni*	S: AGCTGCTTCGCAACTTTCTACGGT AS: TGCACTCTCGGCTGCAAAGTCT	325	30'' a 95ºC, 30'' a 59ºC, 45" a 72ºC
14	pldA [a,d] – fosfolipase A, *C. jejuni*	S: AAGAGTGAGGCGAAATTCCA AS: GCAAGATGGCAGGATTATCA	385	
15	ciaB [a,d] – antígeno de invasão de *Campylobacter*	S: TCATGCGGTGGCATTAGAATGGG AS: GCGACCGATGATAACATCAAGGCT	658	30'' a 95ºC, 30'' a 64ºC, 45" a 72ºC
16	racR [a,d] – regulador de resposta DNA-ligante, *C. jejuni*	S: TGGGGCTTCAAATCGGTGCTGA AS: GCGACCGATGATAACATCAAGGCT	326	
17	dnaJ [a,d] – proteína chaperona DnaJ, *C. jejuni*	S: AGGCTTTGGCTCATCACGTCG AS: GGTCGCTTCACCGCGTATGG	574	
18	SAT [a,c] – autotransportador de toxina secretada, patotipos de *E. coli*	S: TCAGAAGCTCAGCGAATCATTG AS: CCATTATCACCAGTAAAACGCACC	931	30'' a 95ºC, 30'' a 58ºC, 1' a 72ºC

Legend:
[a] Gene cromossomal; [b] Gene plasmidial, [c] PCR comum; [d] PCR Multiplex.
S: iniciador senso; AS: iniciador anti-senso
pb: pares de bases

Table 2: Validated bio-markers from the INCT_Biomedicine and their use in international networks RECODISA and MAL_ED.

Biomarkers	Target gene description	Primers sequence (5´- 3´)	Product (pb)	PCR conditions	Reference
	Gene for diagnosis				
1	*ipaH* [a,c] – *E. coli* enteroinvasiva (EIEC)	S: TGGAAAAACTCAGTGCCTCT AS: CCAGTCCGTAAATTCATTCT	423	30'' a 95ºC, 30'' a 57ºC, 45" a 72ºC	MalEd Project, 2009.
2	*aatA* [b,c] – proteína transportadora anti-agregação, *E. coli* enteroagregativa (EAEC)	S: CTGGCGAAAGACTGTATCAT AS: CAATGTATAGAAATCCGCTGTT	630		
3	*stx1* [a,d] – shiga-toxina 1, *E. coli* enterohemorrágica (EHEC)	S: CAGTTAATGTGGTGGCGAAGG AS: CACCAGACAATGTAACCGCTG	348		
4	*stx2* [a,d] – shiga-toxina 2, EHEC	S: ATCCTATTCCCGGGAGTTTACG AS: ATCCTATTCCCGGGAGTTTACG	584		
5	*eae* [a,d] – intimina, *E. coli* enteropatogênica (EPEC)	S: CCCGAATTCGGCACAAGCATAAGC AS: CCCGGATCCGTCTCGCCAGTATTCG	881		
6	*bfpA* [b,d] – proteína bfpA, EPEC	S: GGAAGTCAAATTCATGGGGGTAT AS: GGAATCAGACGCAGACTGGTAGT	300		
7	*LT* [b,d] – toxina termolábil, *E. coli* enterotoxigênica (ETEC)	S: CACACGGAGCTCCTCAGTC AS: CCCCCAGCCTAGCTTAGTTT	508		
8	*ST* [b,d] – toxina termoestável, ETEC	S: GCTAAACCAGTAGAGGTCTTCAAAA AS: CCCGGTACAGAGCAGGATTACAACA	147		
	Virulence gene				
9	*aap* [b,d] – dispersina, EAEC	S: ATGAAAAAAATTAAGTTTGTTATCTT	356	30'' a 95ºC, 30'' a 55ºC, 1' a 72ºC	Sheik *et al*, 2002.
10	*astA* [b,d] – EAST1, EAEC	AS: GGTCGCGAGTGACGGCTTTGT	112		Piva *et al*, 2003.

Legend:
[a] Gene cromossomal; [b] Gene plasmidial, [c] PCR comum; [d] PCR Multiplex.
S: iniciador senso; AS: iniciador anti-senso
pb: pares de bases

Referrences:

- Sheikh J, Czeczulin JR, Harrington S, et al. A novel dispersin protein in enteroaggregative Escherichia coli. *J Clin Invest* 2002; 110:1329-37.
- Piva IC, Pereira AL, Ferraz LR, et al. Virulence markers of enteroaggregative Escherichia coli isolated from children and adults with diarrhea in Brasilia, Brazil. *J Clin Microbiol* 2003; 41:1827-32.

Table 3: Bio-markers developed in INCT_Biomedicina for diagnosis and assessment of genetic polymorphism.

Biomarkers	Target gene description	Primers sequence (5´- 3´)	Product (pb)	Amplification
	Gene for diagnosis			
1	Análise de polimorfismo de um único nucleotídeo no estudo de Intolerância à Lactose (C/T -13910)	S: GAGTGTAGTTGTTAGACGGAGAC AS: ATCAAACATTATACAAATGCAAC	210	30'' a 95ºC, 30'' a 54ºC, 1' a 72ºC
2	Análise de polimorfismo de um único nucleotídeo para o gene do Receptor *Toll-Like*-5	S: GGTAGCCTACATTGATTTGC AS: GAGAATCTGGAGATGAGGTACCCG	277	30'' a 95ºC, 30'' a 60ºC, 1' a 72ºC

Table 4: Bio-markers validated for the study of polymorphisms in regulatory genes of cytokines.

Biomarkers	Target gene description	Primers sequence (5´- 3´)	Product (pb)	Amplification	Reference
	Gene for diagnosis				
1	Análise de polimorfismo de um único nucleotídeo para o gene de Interleucina 8 humana (IL-8)	S: ACTATATCTGTCACATGGTACTATG AS: CTTATCAAATACGGAGTATGACG	166	30'' a 95ºC, 30'' a 54ºC, 1' a 72ºC	Jiang et al., 2003
2	Análise de polimorfismo de um único nucleotídeo no estudo de Intolerância à Lactose (G/A -22810)	S: AACAGGCACGTGGAGGAGTT AS: CCCACCTCAGCCTCTTGAGT	448	30'' a 95ºC, 30'' a 60ºC, 1' a 72ºC	Bünning et al., 2003

References:

- JIANG, Z. D. *et al.* Genetic susceptibility to enteroaggregative *Escherichia coli* diarrhea: polymorphism in the interleukin-8 promotor region. *J. Infect. Dis.* 2003; 188: 506-511.
- BÜNING, C *et al.* The C/C (-13910) and G/G (-22018) genotypes for adult-type hypolactasia are not associated with inflammatory bowel disease. *Scand J Gastroenterol* 2003; 38:538-542.

Table 5: Bio-markers developed to study transport proteins and joints strong in intestinal barrier function.

Biomarkers	Target gene description	Primers sequence (5´- 3´)	Product (pb)	Amplification
1	Claudina-1	S: TCTACGAGGGACTGTGGATG AS: TCAGATTCAGCAAGGAGTCG	84	20'' a 95ºC, 20'' a 55ºC, 45'' a 72ºC
2	Claudina-2 para camundongo	S: CCCACCACCACCAGCTTAAT AS: GAAATGGCTTCCAGGTCAGC	170	20'' a 95ºC, 20'' a 60ºC, 45'' a 72ºC
3	Claudina-2 para rato	S: AGGACTTCCTGCTGACATCCA AS: TCCACCCACTACAGCCACTCT	154	30'' a 95ºC, 30'' a 60ºC, 1' a 72ºC
4	Zona Ocludens-1 para camundongo	S: GACCATCGCCTACGGTTTGA AS: AGGTCTCGGGGATGCTGATT	116	20'' a 95ºC, 20'' a 60ºC, 45'' a 72ºC
5	Zona Ocludens-1 para rato	S: CTCGCACGTATCACAAGCTGA AS: CCTCAGGATATGGCTCCTTCC	137	30'' a 95ºC, 30'' a 60ºC, 1' a 72ºC
6	Ocludina para camundongo	S: AAGAGCAGCCAAAGGCTTCC AS: CGTCGGGTTCACTCCCATTA	199	20'' a 95ºC, 20'' a 60ºC, 45'' a 72ºC
7	Ocludina para rato	S: AACAGCCCCCTAATGTGGAAG AS: GAGTAGGCCATTGGACTGTCG	112	30'' a 95ºC, 30'' a 60ºC, 1' a 72ºC
8	Beta-catenina para camundongo	S: TGGTGTCTGCCATTGTACGC AS: CCACTGGTGACCCAAGCATT	165	20'' a 95ºC, 20'' a 60ºC, 45'' a 72ºC
9	Beta-catenina para rato	S: CTGGCAGCAGCAATCTTACCT AS: AAAGGACTTGGGAGGTGTCCA	119	30'' a 95ºC, 30'' a 60ºC, 1' a 72ºC
10	Transportador de peptídeo intestinal-1 (PEPT-1) para rato	S: CCTGAAGAAGATGACCGTTGG AS: GCTGGGGAAGACTGGAAGAGT	103	30'' a 95ºC, 30'' a 60ºC, 1' a 72ºC
11	GAPDH para camundongo	S: AGCCTCGTCCCGTAGACAAA AS: TGAATTTGCCGTGAGTGGAG	183	20'' a 95ºC, 20'' a 60ºC, 45'' a 72ºC
12	GAPDH para rato	S: GTTACCAGGGCTGCCTTCTCT AS: AACTTGCCGTGGGTAGAGTCA	116	30'' a 95ºC, 30'' a 60ºC, 1' a 72ºC
13	Transportador de peptídeo intestinal-1 (PEPT-1) para coelho	S: GGGAGTCTGCTGTCCACAATC AS: GTACATCCCACTGCCGATGAT	153	20'' a 95ºC, 20'' a 64,6ºC, 45'' a 72ºC
14	Co-transportador Sódio-Glicose intestinal para coelho (SGLT-1)	S: CTGACTGGGTTCGCTTTTCAC AS: GCATCTCGGAAGATGTGGAAG	155	20'' a 95ºC, 20'' a 65ºC, 45'' a 72ºC

15	GAPDH para coelho	S: GCCGTGGGCAAGGTCATCCC AS: GCAGCTTTCTCCAGGCGGCA	113	20'' a 95ºC, 20'' a 64,6ºC, 45'' a 72ºC

Table 6: Bio-markers validated for the study of intestinal glutamine transport.

Biomarkers	Target gene description	Sequência dos iniciadores (5´- 3´)	Product (pb)	Amplification	Reference
	Gene for diagnosis				
1	Transportador de aminoácidos intestinal (SN-1) para coelho	S: GGCAGGGGTTTCCTACAGA AS: CGATGTCTTCCCCTCGAA	69	20'' a 95ºC, 20'' a 62,2ºC, 45'' a 72ºC	Talukder *et al*., 2008
2	Transportador de aminoácidos intestinal (SN-2) para coelho	S: CTGGGACAGAGGGCATTC AS: CGGATTTGATGATGAACAGGT	106	20'' a 95ºC, 20'' a 62,2ºC, 45'' a 72ºC	

References:

- Talukder *et al.* Functional characterization, localization, and molecular identification of rabbit intestinal N-amino acid transporter. *Am J Physiol Gastrointest Liver Physiol* 2008; 294: G1301–G1310.

Table 7: Bio-markers developed for the study of receptor guanylyl cyclases and natriuretic peptide C.

Biomarkers	Target gene description	Primers sequence (5´- 3´)	Product (pb)	Amplification
1	Receptor de Guanilato ciclase A de rato (GC-A)	S:GAACCGAAGCTTCCAAGGTG AS: GTGGATATCCCAGAGGCCAGT	218	30'' a 95ºC, 30'' a 58ºC, 1' a 72ºC
2	Receptor de Guanilato ciclase B de rato (GC-B)	S: CATCTGCATCGTCACCGAGT AS: TCCACCACGCAGTTAGAGGAC	186	30'' a 95ºC, 30'' a 59ºC, 1' a 72ºC
3	Receptor de Guanilato ciclase C de rato (GC-C)	S: GGCGGGATACAATCCAGAGAG AS: ACGGTGCCGTAGAACTTGGTC	166	30'' a 95ºC, 30'' a 63ºC, 1' a 72ºC
4	Receptor de Peptídeo Natriurético C de rato (NPR-3)	S: CCTACAATTTCGACGAGACCAAA AS: TCGCTCACTGCCCTGGAT	201	30'' a 95ºC, 30'' a 59ºC, 1' a 72ºC
5	RNA ribossômico cadeia 18S de rato (18S rRNA)	S: ACATCCAAGGAAGGCAGCAG AS: GCTGGAATTACCGCGGCTG	179	30'' a 95ºC, 30'' a 60ºC, 1' a 72ºC

Table 8: Bio-markers developed for the diagnosis of bacterial resistance.

Biomarkers	Target gene description	Primers sequence (5´- 3´)	Product (pb)	PCR conditions
	Gene for diagnosis			
1	*katG* - catalase-peroxidase, *Mycobacterium tuberculosis*	S: GTCGGCGGTCACACTTTC AS: GCTACCACGGAACGACGAC	86	30'' a 95ºC, 30'' a 64ºC, 1' a 72ºC
2	*rpoB* – subunidade beta da RNA polimerase direcionada ao DNA, *M. tuberculosis*	S: TACGGTCGGCGAGCTGATCC AS: TACGGCGTTTCGATGAACC	165	30'' a 95ºC, 30'' a 61ºC, 1' a 72ºC
3	*inhA* - enoil-(acil proteína carreadora) redutase, *M. tuberculosis*	S: TGCTCGAACTCGACGTGCAA AS: CGAAGCATACGAATACGCCGA	209	30'' a 95ºC, 30'' a 62ºC, 1' a 72ºC