Travelan

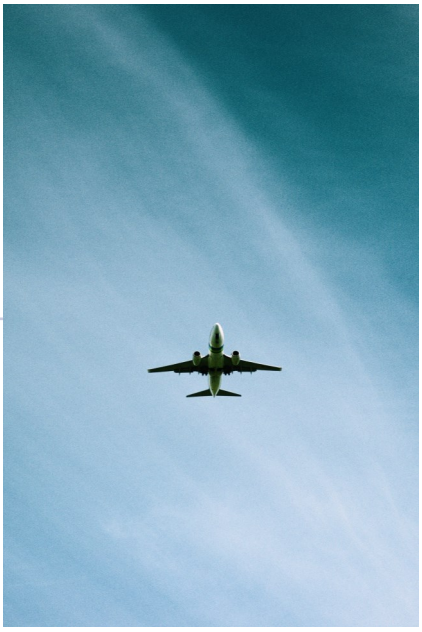
Scientific Review

Travelan® is an orally administered passive immunotherapy that prophylactically reduces the likelihood of contracting travelers' diarrhea. Travelan® is a highly purified tabletised preparation of hyperimmune bovine colostrum antibodies and other factors, which when taken with meals bind to diarrhoea-causing bacteria and prevent colonisation and the pathology associated with travelers' diarrhea (TD).

Travelan® is an effective, inexpensive, non-prescription prophylactic alternative to antibiotic therapy for travelers' diarrhea, which avoids the inconvenient adverse events and bacterial resistance issues associated with antibiotic treatment.

In Australia Travelan® is approved by the Therapeutic Goods Administration (TGA) as a listed medicine in the Australian Register of Therapeutic Goods (AUST L106709) and is indicated to reduce the risk of traveller's diarrhoea and associated symptoms of minor gastrointestinal disorders.

In the USA Travelan® is sold as a dietary supplement in accordance with section 403 (r)(6) of the Federal Drug Administration (FDA).



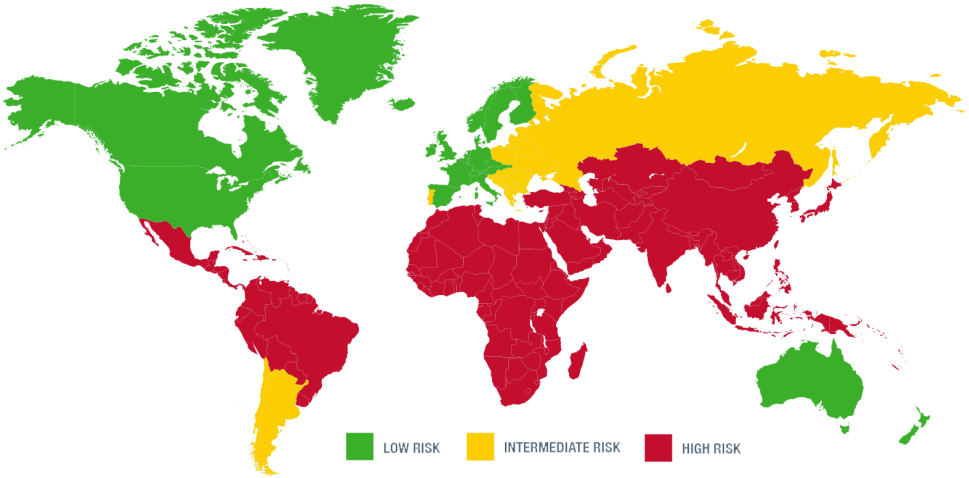
The research behind Travelan®

Travelan® is the most tested and scientifically proven over-the-counter, preventative travelers' diarrhea treatment available and the result of meticulous scientific development. Several controlled clinical studies have been carried out that confirm Travelan®'s effectiveness in reducing travelers' diarrhea, its safety, tolerability and its mechanism of action. These stringent double-blind, randomised, placebo controlled, phase I and 2 clinical trials were conducted in Europe and the USA, and were fully documented using Good Clinical Practice (GCP) and approved by the Food and Drug Administration in the USA and the Therapeutic Goods Administration in Australia. Each of these trials were conducted in specific clinical trial units by highly reputable clinical research scientists with prior experience in this area.

Travelers' diarrhea (TD)

Travelers' diarrhea is a gastro-intestinal infection with symptoms that include loose, watery (and occasionally bloody) stools, abdominal cramping, bloating, and fever, when travelling to unfamiliar destinations. Entropathogenic bacteria are responsible for most cases, with enterotoxigenic Escherichia coli (ETEC) playing a dominant causative role. Campylobacter spp. are also responsible for a significant proportion of cases. The more serious infections with Salmonella spp., the bacillary dysentery organisms belonging to Shigella spp., and Vibrio spp. (the causative agent of cholera) are often confused with travelers' diarrhea as they may be contracted while travelling and initial symptoms are often indistinguishable.

Medium to high risk areas for travelers' diarrhea



Clinical Studies by the US Defense Force, 2017

For the past 20 years, the US Department of Defense has been searching for a vaccine to prevent travelers' diarrhea. During this time they have tested numerous different products without success.

In 2017, a study conducted by the US Army branch of the Walter Reed Army Institute of Research found that Travelan® could provide the answer. The extensive project researched the inter and intra-species reactivity of Travelan® across 60 isolates each of Campylobacter spp, ETEC, and Shigella spp. collected from infected Army personnel in Bhutan, Cambodia, Nepal and Thailand over a 20 year period. **The results revealed that Travelan® reacted with every single one of the 180 bacteria samples collected.**

Table 1. A sample of the bacteria strains collected from Army personnel.

Campylobacter	Shigella
Campylobacter coli	Shigella bodydii
Campylobacter jejuni subsp. doylei	Shigella boydii 2
Campylobacter jejuni subsp. Jejuni	Shigella boydii 18
Campylobacter upsaliensis	Shigella dysenteriae 2
	Shigella dysenteriae 4
Enterotoxigenic Escherichia coli	Shigella dysenteriae 12
	Shigella flexneri
Enterotoxigenic Escherichia coli (ETEC) LT	Shigella flexneri 1b
Enterotoxigenic Escherichia coli (ETEC) LT, ST	Shigella flexneri 2 variant (II: 3,4,7,8)
Enterotoxigenic Escherichia coli (ETEC) LT, STIa	Shigella flexneri 2a
Enterotoxigenic Escherichia coli (ETEC) LT, STIa, STIb	Shigella flexneri 3a
Enterotoxigenic Escherichia coli (ETEC) LT, STIb	Shigella flexneri 4
Enterotoxigenic Escherichia coli (ETEC) STIa	Shigella flexneri 4av
Enterotoxigenic Escherichia coli (ETEC) STIb	Shigella flexneri 6
	Shigella sonnei

This independent study clearly demonstrates that Travelan® is immune-reactive with all ETEC strains tested including strains that are not present in the immunising vaccines and cross-reactive with every Campylobacter and Shigella isolate tested. These impressive results, together with other similar studies strongly suggest Travelan® is an effective immunoprophylactic for ETEC-mediated travelers' diarrhea and the more serious enteric infections caused by Campylobacter spp and Shigella spp.

Immuron's clinical collaborators have carried out a study aimed at assessing the prophylactic protective qualities of powdered hyper immune colostrum incorporated into Travelan®. This study was published in the Scandinavian Journal of Gastroenterology in 2011.

Two separate randomised, double-blind placebo-controlled trials involving 90 healthy adult volunteers were performed. In the first of the studies, 30 patients were randomised into two groups of 15. Volunteers in each group were either prophylactically treated with either placebo tablets or tablets containing 400 mg of colostrum protein, taken three times daily with a bicarbonate buffer. All patients were challenged with a 1 x 10^9 CFU of ETEC strain O78: H11 H10407, which was also present in one of the vaccine preparations used to generate the hyperimmune colostrum (see table 2). This regimen conferred **90.9% protection against diarrhoea** in the group receiving the active preparation compared with the placebo group.

Table 2. Summary of results of study 1: prophylactic efficacy of hyperimmune bovine colostrum powder (400 mg doses) and placebo against infection with enterotoxigenic E. coli strain, H10407.

	Treatment group		
	Placebo	Colostrum	p^*
Number of volunteers	15	15	
Number of volunteers with diarrhea	11 (73%)	1 (7%)	0.0005
Number of diarrheal stools/volunteer (mean ± SEM)	4.4 ± 0.9	0.4 ± 0.4	0.0004
Mean number of diarrheal stools per volunteer with diarrhea (mean and range)	6 (2–8)	6 (6)	NS
Abdominal pain	5 (33%)	0 (0%)	0.04
ETEC H10407 isolated from feces after challenge	15 (100%)	12 (80%)	NS

*Fisher's exact test or Student's *t*-test (two-tailed) as appropriate. NS, not significant.

Interestingly, ETEC was isolated from almost 100% of subjects after the challenge, irrespective of their placebo or colostrum treatment status, indicating that the antibodies in the hyperimmune colostrum powder were not killing the bacteria *per se*, but preventing their colonisation and subsequent pathology.

Table 3. Overall results from a clinical study assessing the efficacy of Travelan® hyperimmune colostrum powder in ETEC challenge (Otto et al 2011).

	Treatment group			
	Group 1: Placebo tid	Group 2: Colostrum 400 mg tid + buffer	Group 3: Colostrum 200 mg tid	Group 4: Colostrum 400 mg tid
Number of volunteers	14	14	14	15
Number of volunteers with diarrhea	12 (86%)	2 (14%), $p = 0.0004$*	5 (36%), $p = 0.02$	3 (20%), $p = 0.007$
Number of diarrheal stools/volunteer (mean ± SEM)	3.9 ± 0.8	0.5 ± 0.3, $p = 0.0005$	1.8 ± 0.8, $p = 0.07$	0.9 ± 0.5, $p = 0.003$
Mean number of diarrheal stools per volunteer with diarrhea (mean and range)	5 (3–10)	3.5 (3–4)	5 (2–7)	4.7 (2–7)
Abdominal pain	5 (36%)	0 (0%), $p = 0.04$	2 (14%), $p = 0.04$	0 (0%), $p = 0.02$
ETEC H10407 isolated from feces after challenge	12 (86%)	14 (100%)	14 (100%)	12 (80%)

Proven Travelan® Advantages

In addition to the pivotal US Army study and clinical trials, Immuron and its respected collaborators have invested significant intellectual and financial capital in unambiguously demonstrating the scientific data underpinning the advantages of its Travelan® technology. Their findings include;

- Travelan contains significantly higher concentrations of innate and adaptive immune products than milk, and is immunoreactive with many strains of enteropathogenic *E.coli* and *E.coli* heat labile enterotoxin (Sears et al. Clin Vaccine Immunol. 2017;24(8)).
- Travelan® antibodies react with both flagella and surface 'O polysaccharide' and 'lipid A' LPS core region of all the serotypes included in the ETEC vaccine. They also react with bacterial flagella and Colonisation Factor Antigens.
- Travelan® antibodies cross react with both similar antigens of ETEC serotypes **not** included in the ETEC vaccine.
- Travelan® antibodies even cross react with antigens of other Gram-negative enteropathogenic bacteria, e.g. Salmonella spp, Vibrio spp, Yersinia spp and Klebsiella spp.
- Travelan® antibodies agglutinate ETEC in liquid suspension and significantly reduce their ability to adhere to enterocytes (CaCo2 cells).
- Travelan® antibodies significantly reduce adherence of CFA/I-producing ETEC strains to enterocytes (CaCo2 cells)
- Travelan® antibodies have substantially greater reactivity against purified ETEC antigens than IgG purified from non-immune colostrum powder.

Collectively, these results show that Travelan® antibodies interfere with the processes (motility and adherence) necessary for bacterial colonization and establishment of a clinical enteric infection. It is highly likely that these combined effects contribute significantly to Travelan®'s TD-protective effects.

Travelan® antibodies cross react with both protein and LPS components from several species of enteropathogenic gram-negative bacteria. LPS, flagellin and CFA antigens have unique antigenic qualities that allow the identification of individual bacterial isolates down to the precise serovar level, and that each genus, species and serovar has its own unique antigenic signature. Equally, there are invariant regions of the antigens (e.g. the Lipid-A core of LPS) that are antigenically conserved across all strains and species etc. Many of the bovine antibodies incorporated into Travelan® are generated by the ***invariant antigenic regions*** and therefore do not discriminate between ETEC strains, or ETEC and Salmonella and Campylobacter or Shigella, and are considered truly cross-reactive and presumably account for the cross-species protection afforded by Travelan®.

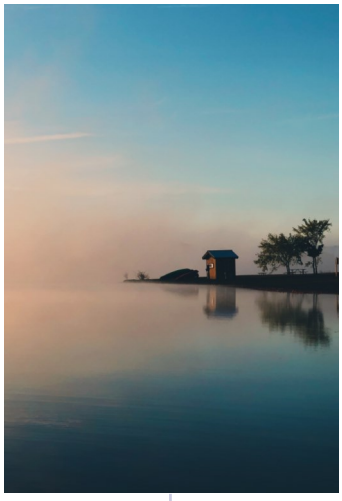


Table 4. Immunising ETEC serotypes and strains used in the generation of Travelan® immunotherapy

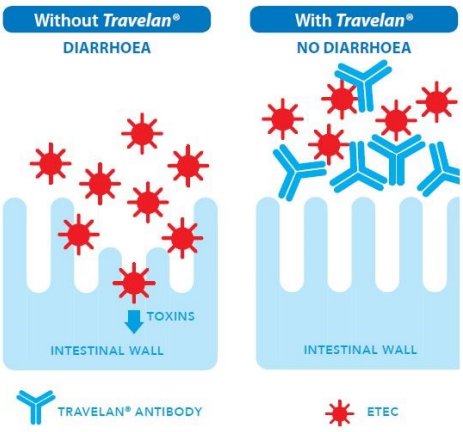
Serotype	Strain	Serotype	Strain	Serotype	Strain	Serotype	Strain
ETEC O6: H16	B2C	ETEC O27: HR	C1067-77	ETEC O114: H21	E20738/0	ETEC O148: H28	B7A
ETEC O8: H19	C55 3/3c3	ETEC O63: H-	PE 673	ETEC O115: H-	PE 724	ETEC O153: H12	E8772/0
ETEC O15: H4	PE 595	ETEC O78: H11	H10407	ETEC O128: H21	EI 37-2	ETEC O159: H-	PE 768
ETEC O25: H42	E11881A						

Travelan® is a formulation of bovine antibodies derived from hyperimmune colostrum. Gravid Holstein Friesian and Jersey cows are vaccinated with a highly a purified preparation of 13 ETEC serotypes. The vaccine contains three important pathogenic and antigenic determinants; lipopolysaccharide (LPS), flagella, and colonisation factor antigen (CFA), which collectively play roles in bacterial membrane stability, immune evasion, motility, and adherence.

Vaccinated cows generate a powerful immune response with a high concentration of vaccine-specific antibodies secreted into the anti-natal colostrum. The first milking colostrum is collected within twelve hours of calving and active ingredient containing high titre anti-ETEC antibodies is prepared, lyophilised, milled and processed into the oral dosage form. A majority of the antibodies found in the hyperimmune colostrum are bovine IgG_1 isotype. All processes conform to cGMP criteria.

The antibodies generated against the O-polysaccharide component of LPS are often serotype specific, but anti-lipid-A antibodies that recognise the core region are cross-reactive between serotypes and even between species. Anti-LPS and anti-CFA antibodies agglutinate bacteria and interfere with bacterial adherence, thereby preventing their colonisation of the gut luminal surface. Anti-flagellin antibodies bind to and inactivate flagella, preventing bacterial motility and hence the bacterium's ability to colonise the gut.

Travelan® mode of action



The clinical studies conducted on Travelan® clearly demonstrate that the hyperimmune colostrum antibodies in Travelan® bind to and functionally inhibit critical antigens present on the surface of the enteropathogenic bacteria. Travelan® antibodies conferred impressive protection against ETEC pathology in a randomised double-blind challenge study. Taken together these results demonstrate the benefit of prophylactic treatment with Travelan® during travel to TD-endemic regions of the world.

Refs
1. Sears et al Clin Vaccine Immunol. 2017 Aug 4;24(8)
2. Otto et al Scandinavian Journal of Gastroenterology 2011;46:862 - 868