

## Clinical Trial at Kitasato Institute, Japan

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## **Summary**

Excessive oxidation reaction in living bodies induces sedimentation of blood cells such as erythrocytes and leukocytes on the intima of the blood vessels, contribution to occurrence of arteriosclerosis. The major causes are attributed to activation of vascular endothelial cells and stimulation of platelet agglutination due to lipid peroxides. These are usually caused by excessive intake of lipid, influences of intestinal environment or stresses. The following clinical study was conducted with healthy volunteers using “preB food,” which is classified as a fermented vegetable food, and is recognized to affect the intestinal bacterial flora, which is conducive to controlling the intestinal environment. PreB is a kind of supplemental health food containing over 55 kinds of grains, vegetables, fruits and wild herbs. Hematological and biochemical tests centering on hepatic function assessment were performed before and after the ingestion of the test substance.

Together with molecular biological analyses of the intestinal bacterial flora, and immunological analyses mainly on T cells, the presence or absence of the possible influences of preB on the body was investigated.

The results confirmed that the ingestion of preB does not provide any influences on either hepatic functions or changes in lipid metabolisms. In addition, it indicated that in more than half the volunteer subjects there was a change in the intestinal environment. It suggested that preB would not cause any safety-related problems, and at the same time exhibit positive effects on the intestinal environment.

## **Introduction**

Oxidated substances produced by aging and stresses in the body are involved in the excessive accumulation of hyper-oxidated lipids, hepatic disorders and arteriosclerosis. They also possess the risk of inducing carcinogenicity through DNA damage in the cells. Oxidated substances in the body exist as metabolites, which are derived from the biological defense mechanisms. In addition, however, they accumulate excessively in the body due to westernized dietary habits or stresses, and currently attention has been paid to them as one of the causes of the stimulation of aging along with the above-stated diseases. In this connection, excessive ingestion of lipids is responsible for the onset of lifestyle-related diseases including arteriosclerosis and hypertension as their direct causes whereas the intestinal environment is also designated as one of the indirect factors to incur these diseases. For the purpose of improving the intestinal environments, probiotic products, mainly comprising lactic acid bacteria beverages, have currently been employed. In this relation, development of functional foods exerting normalization of the intestinal environment and anti-oxidation effects against oxidants is highly expected in terms of their usefulness as the QOL improving supplements.

PreB used in the present study is designated as a liquid-type health food containing vegetable-origin lactobacillus and dietary fiber. PreB also contains other useful ingredients such as Lutein, which is a yellow pigment contained in green and yellow vegetables and is known to exert anti-oxidation effects, plus it represents carotenoids (pigment group) like  $\beta$ -carotene and lycopene. In the present study, efficacy confirmation and the safety verifications were conducted using preB in healthy volunteers as the study subjects.

**Title** Safety study on preB

**Study Number** 00802

**Purpose:** To re-confirm the safety of preB by assessing the hepatic function test items, and also to analyze the possible influences on the intestinal environment.

**Sponsor**

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**Study Director:** Suzuki Tatsuo

Date of study initiation: May 14, 2008  
Date of study completion: September 9, 2008  
Date of preparation of the final report: September 17, 2008

## Methods

### 1. Materials

#### 1.1 Test substance

Name: PreB

Code number:

Reference: A kind of supplemental health food containing over 55 kinds of grains, vegetables, fruits and wild herbs.

#### 1.2 Test subjects

Animal species: Human

Strain: Yellow race of Asian descent

Gender: Both sexes

Age: Healthy volunteers of 20 years old or older

Enrolled number: 10 Males and 20 females, 30 in total.

### 2. Test methods

#### Screening of the test subjects

On 40 candidate test subjects (15 males and 25 females) who showed normal ranges of respective hematological test values (within 3SD from the highest and the lowest limit of the normal values), but took no medical drugs continuously (daily); physician's inquiry, physical determination, urine collection and blood collection were conducted to perform various tests including the general hematological tests. Based on the test results, 10 males and 20 females were selected as the test subjects.

#### 2.1 Test methods

To each of the 10 male and 20 female healthy test volunteers, one dose (15grams) of the test substance was given after breakfast and after supper (30 grams per day).

The administration period was designated as one month (28 days). Before commencement, and at the completion of administration, urine samples, blood samples and fecal samples were collected. These were used for various tests such as the general hematological tests, intestinal bacterial flora tests and immunological tests. Simultaneously, the physician's inquiry was conducted at the time of the commencement of the test and at the completion of the test.

### 2.3 Test items

Physical determination: Height, body weight, body fat mass (only at the initiation of the test and at the completion of the test), and blood pressure.

Physician's inquiry: Gastrointestinal symptoms and general physical conditions

Full set of hepatic function tests: AST, ALT and ALP (Enzymatic method), etc.

Full set of renal function tests: Creatinine, etc.

Hemoglobin, cholesterol, triglyceride

Determination of CD2/CD20/CD4/CD8

Determination of plasma cytokines

Determination of bacterial flora in feces

## Results

### 3.1 Influences of administration of preB on blood corpuscle cells in blood

In the male subjects, WBC values were  $69.8 \pm 14.63/\times 10^2 \mu\text{L}$  before initiation of the study and  $63.50 \pm 8.75/\times 10^2 \mu\text{L}$  at the end of the study (Figure 1 and Table 1).

RBC values were  $49.09 \pm 2.66/\times 10^5 \mu\text{L}$  before initiation of the study and  $47.52 \pm 1.99/\times 10^5 \mu\text{L}$  at the end of the study (Figure 1 and Table 1).

Hb values were  $15.20 \pm 0.88 \text{ g/dL}$  before commencement of the study and  $14.77 \pm 0.78 \text{ g/dL}$  at the end of the study (Figure 1 and Table 1).

Hematocrit values (Hbt) were  $44.05 \pm 2.38\%$  before start of the study and  $42.90 \pm 1.89\%$  at the end of the study (Figure 1 and Table 1).

Platelet counts (Plt) were  $26.87 \pm 4.46/\times 10^4 \mu\text{L}$  before commencement of the study and  $25.29 \pm 3.52/\times 10^4 \mu\text{L}$  at the end of the study (Figure 1 and Table 1).

In female subjects, WBC values were  $64.10 \pm 12.47/x 10^2 \mu\text{L}$  before initiation of the study and  $61.65 \pm 12.92/x 10^2 \mu\text{L}$  at the end of the study (Figure 1 and Table 1).

RBC values were  $41.77 \pm 3.56/x 10^5 \mu\text{L}$  before initiation of the study and  $40.50 \pm 3.25/u 10^5 \mu\text{L}$  at the end of the study (Figure 1 and Table 1).

Hb values were  $12.54 \pm 1.36 \text{ g/dL}$  before commencement of the study and  $12.24 \pm 1.16 \text{ g/dL}$  at the end of the study (Figure 1 and Table 1).

Hematocrit values (Hbt) were  $37.12 \pm 3.41\%$  before start of the study and  $36.13 \pm 2.82\%$  at the end of the study (Figure 1 and Table 1).

Platelet counts (Plt) were  $28.55 \pm 5.38/x 10^4 \mu\text{L}$  before commencement of the study and  $28.46 \pm 5.55/x 10^4 \mu\text{L}$  at the end of the study (Figure 1 and Table 1).

As stated above, the findings in all the test items concerning the blood corpuscle cells in blood were within the normal ranges irrespective of males or females.

### 3.2 Influences of administration of preB on hepatic functions

In male subjects, AST values were  $25.55 \pm 7.52 \text{ IU/L}$  before start of the study and  $25.30 \pm 9.65 \text{ IU/L}$  at the end of the study (Figure 2 and Table 2).

ALT values were  $35.20 \pm 20.86 \text{ IU/L}$  before beginning of the study and  $31.60 \pm 16.08 \text{ IU/L}$  at the end of the study (Figure 2 and Table 2).

ALP values were  $224.00 \pm 64.15 \text{ IU/L}$  before commencement of the study and  $222.60 \pm 75.78 \text{ IU/L}$  at the end of the study (Figure 2 and Table 2).

In female subjects, AST values were  $17.15 \pm 2.68 \text{ IU/L}$  before start of the study and  $17.05 \pm 2.74 \text{ IU/L}$  at the end of the study (Figure 2 and Table 2).

ALT values were  $14.25 \pm 3.29 \text{ IU/L}$  before beginning of the study and  $13.55 \pm 4.71 \text{ IU/L}$  at the end of the study (Figure 2 and Table 2).

ALP values were  $164.25 \pm 31.81 \text{ IU/L}$  before commencement of the study and  $168.60 \pm 30.99 \text{ IU/L}$  (Figure 2 and Table 2).

As stated above, the findings in all the test items regarding the hepatic function test values except AST values in male subjects were within the normal ranges irrespective of sexes.

### 3.3 Influences of administration of preB on lipid metabolism

In male subjects, high-density lipoprotein cholesterol values (HDL-CHO) were  $4550 \pm 6.31$  mg/dL before commencement of the study and  $48.55 \pm 8.18$  mg/dL at the end of the study (Figure 3 and Table 2).

Low-density lipoprotein cholesterol values (LDL-CHO) were  $121.50 \pm 35.20$  mg/dL before beginning of the study and  $123.00 \pm 29.71$  mg/dL at the end of the study (Figure 3 and Table 2).

Triglyceride values were  $196.20 \pm 8.4$  mg/dL before start of the study and  $171.20 \pm 160.34$  mg/dL at the end of the study (Figure 3 and Table 2).

In female subjects, high-density lipoprotein cholesterol values (HDL-CHO) were  $67.40 \pm 14.08$  mg/dL before commencement of the study and  $63.00 \pm 11.47$  mg/dL at the end of the study (Figure 3 and Table 2).

Low-density lipoprotein cholesterol values (LDL-CHO) were  $103.90 \pm 15.44$  mg/dL before beginning of the study and  $101.70 \pm 18.81$  mg/dL at the end of the study (Figure 3 and Table 2).

Triglyceride values were  $81.10 \pm 52.28$  mg/dL before start of the study and  $82.65 \pm 47.46$  mg/dL at the end of the study (Figure 3 and Table 2).

As stated above, the findings in all the test items concerning the test values associated with lipid metabolism except triglyceride values in male subjects were within the normal ranges irrespective of males or females.

#### 3.4 Effects of PreB intake on renal function

In male subjects, serum creatinine (CRA) levels were  $0.84 \pm 0.08$  mg/dL before starting the trial test and  $0.81 \pm 0.09$  mg/dL at completion of the trial test (Figure 4 and Table 2).

The haemoglobin A1c (HbA1c) levels were  $5.21 \pm 0.32$  mg/dL before starting the trial test and  $5.23 \pm 0.27$  mg/dL at completion of the trial test (Figure 4 and Table 2).

In female subjects, levels of serum creatinine level were  $0.64 \pm 0.10$  mg/dL before starting the trial test and  $0.65 \pm 0.09$  mg/dL at completion of the trial test (Figure 4 and Table 2).

The haemoglobin A1c (HbA1c) levels were  $5.07 \pm 0.29$  mg/dL before starting the trial test and  $5.10 \pm 0.28$  mg/dL at completion of the trial test (Figure 4 and Table 2).

As described above, levels associated with renal function are within the normal range in all test items regardless of sex.

### 3.5 Effects of PreB intake on the immune system

The leukocyte fraction was prepared from the blood, and the lymphocyte fraction was analyzed following multistaining by CD2, CD20, CD4 and CD8 antibodies. The forward-scattering light and the side-scattering light were applied to select polymorphonuclear leukocytes in the lymphocyte fraction. As shown in the Figure 5, Figure 6 and Table 3, no difference was found in the absolute count of T cells, B cells, CD4 positive T cells and CD8 positive T cells between the male subjects and the female subjects, and between at the time of starting and completion of the trial test.

No difference was found in TNF-alpha and IFN-gamma, which were the indicators of cell-mediated immunodominance and IL-10, which were the indicators of humoral immunodominance between the male and female subjects between the time of the start of the trial test and the completion of the trial test (Figure 6 and Table 3).

### 3.6 Evaluation with Visual Analogue Scale (VAS)

Utilizing the visual analogue scale (VAS), constipation, rough skin and stiff shoulders were measured to evaluate effects of ingesting PreB on the body. It was found that the number of instances of constipation, rough skin and stiff shoulders were markedly improved at the completion of the trial test in all subjects compared with the number of instances recorded before the start of the trial test.

### 3.7 Changes in the enteral environment by PreB intake

Changes in the intestinal bacterial flora by PreB intake were evaluated by a simultaneous molecular analysis. The analyzing method was invented by the Department of Microbiology, School of Medicine, University of Occupational

and Environmental Health, and was the application of the fingerprint method using bacteria-derived DNA in the stool. Bacteria in the stool of the 30 subjects were identified from the 2667 clone before starting the trial test and from the 2525 clone after completion of the trial test.

Major components of the intestinal bacterial flora before starting the trial test were the Clostridium species, Ruminococcus species, Faecalibacterium species and Prevotella species (Table 4). Other, components of the intestinal bacterial flora at completion of the trial test were Bacteroides species, Streptococcus species, Faecalibacterium species and Prevotella species (Table 4).

### **Discussion**

Lipid peroxide is one of the oxidized substances which are by-products of cellular metabolism as well as active oxygen, free radicals or superoxides etc. in vivo. The oxidized substances cause oxidization of lipids, and also arteriosclerosis. The oxidized substances are produced by stress from modern life and excessive consumption of calorie-rich food etc. These are separate from the oxidized substances produced by normal immune response and aging. Injuries in normal cells caused by the oxidized substances are considered to increase the acceleration of aging. In addition, the excessive oxidized substances that are produced cause arteriosclerosis because of abnormal metabolism of the oxidized substances, oxidization and cell injuries in cell membranes and nuclear membranes, and have risks of inducing various diseases.

A function of 'functional foods' reduces these excessively produced oxidized substances and has been extensively used as an adjunct to improve daily life. PreB used in this trial test is a health supplement, which contains over 55 kinds of cereals, vegetables, fruits and wild herbs etc. The product contains vegetable origin lactic acid bacteria and lutein, which have an antioxidative effect.

The trial test was conducted to confirm efficacy and safety of PreB in healthy volunteers.

PreB was analyzed for its effects on the body. Results showed that levels of ALT and ALP were within the normal range (the normal range of ALT level: 5 -45 IU/ml and the normal range of ALP level: 100 -325 IU/mL) in both male subjects and in female subjects according to a liver function test when subjects continuously ingested PreB for 1 month. The mean levels of AST were higher in the male subjects than in the female subjects (the normal range of AST level was 10 -40 IU/L), which was a deviation occurred in a male subject who was found to have liver function abnormality. The liver function abnormality was not attributed to the trial test substance but was affected by the subject's diet.

In lipid metabolism, both levels of high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were within the normal range (the normal range of high density lipoprotein cholesterol level: 45 -95 mg/dL, the normal range of low-density lipoprotein-cholesterol level: 65 -139 mg/dL) regardless of sex. Values of triglyceride in the male subjects were higher than the normal upper limit (the normal upper limit of triglyceride: 30 -149 mg/dL). It was confirmed that the high value of triglyceride in the male subjects was caused by the high value of the same male subject whose AST value was the deviation. It was not affected by the trial test substance. Then, for lipids metabolism, creatinine clearance and HbA1c levels which were used as indicators were analyzed for effects on renal function. The values were within the normal range (the normal range of creatinine level: 0.61 -1.04 mg/dL in the male subjects and 0.47 -0.79 mg./dL in the female subjects; HbA1C: 4.0 -55 mg/dL) regardless of sex. According to the above results, it was considered that PreB had no safety problem.

PreB had no effects on the immune system such as white blood cell counts, lymphocyte counts, T cell counts and B cell counts. In addition, there was no change in all subjects in IL-10 which was the indicator of humoral immunodominance before and after the trial test. No difference was found in the mean levels of TNF-alpha and IFN-gamma which were the indicators of cell-mediated immunity. However, a tendency was noted that they were higher at the

completion of the trial test compared with the values before the trial test in more than half subjects (attached Figure 2 and Figure 3). For this reason, it was inferred that activation of macrophages and neutrophils caused by the changes in the intestinal bacterial flora might be involved in the high value.

No change was found in the intestinal bacterial flora, including *Faecalibacterium* species and *Prevotella* species, which were the major components before the trial test and at completion of the trial test. However, *Clostridium* and *Ruminococcus*, which were dominant before the trial test were decreased at the completion of the trial test (from 4.9% to 2.8% and from 8.5% to 4.4%, respectively). The extent of decrease was approximately 50%, and types of bacteria were also reduced to half or changed (Table 4). *Bacteroides* species and *Streptococcus* species were increased at completion of the trial test. These changes were considered to be caused by effects of PreB. Also, these results might be related to aforementioned increase of TNF-alpha and IFN-gamma levels, which meant the activation of macrophages and neutrophils. In addition, it was inferred that the changes in the enteral environment might be involved in improvement of constipation and relief of rough skin according to the VAS evaluation.

Since the healthy volunteers in this trial test lived their normal lives, dramatic changes were not noted in immunocytes and the enteral environment. However, the usefulness of PreB seems to have been revealed by conducting a test on small-sized low-density lipoprotein and a challenge test.

### **Conclusion**

- The safety of PreB was confirmed by the trial test of PreB intake (for 28 days) in healthy volunteers.
- No effects of the PreB ingestion were found in the immunocytes (lymphocytes).
- Changes were found in the intestinal bacterial flora, which were caused by the ingestion of PreB.

Figure 1: Hematologic Data

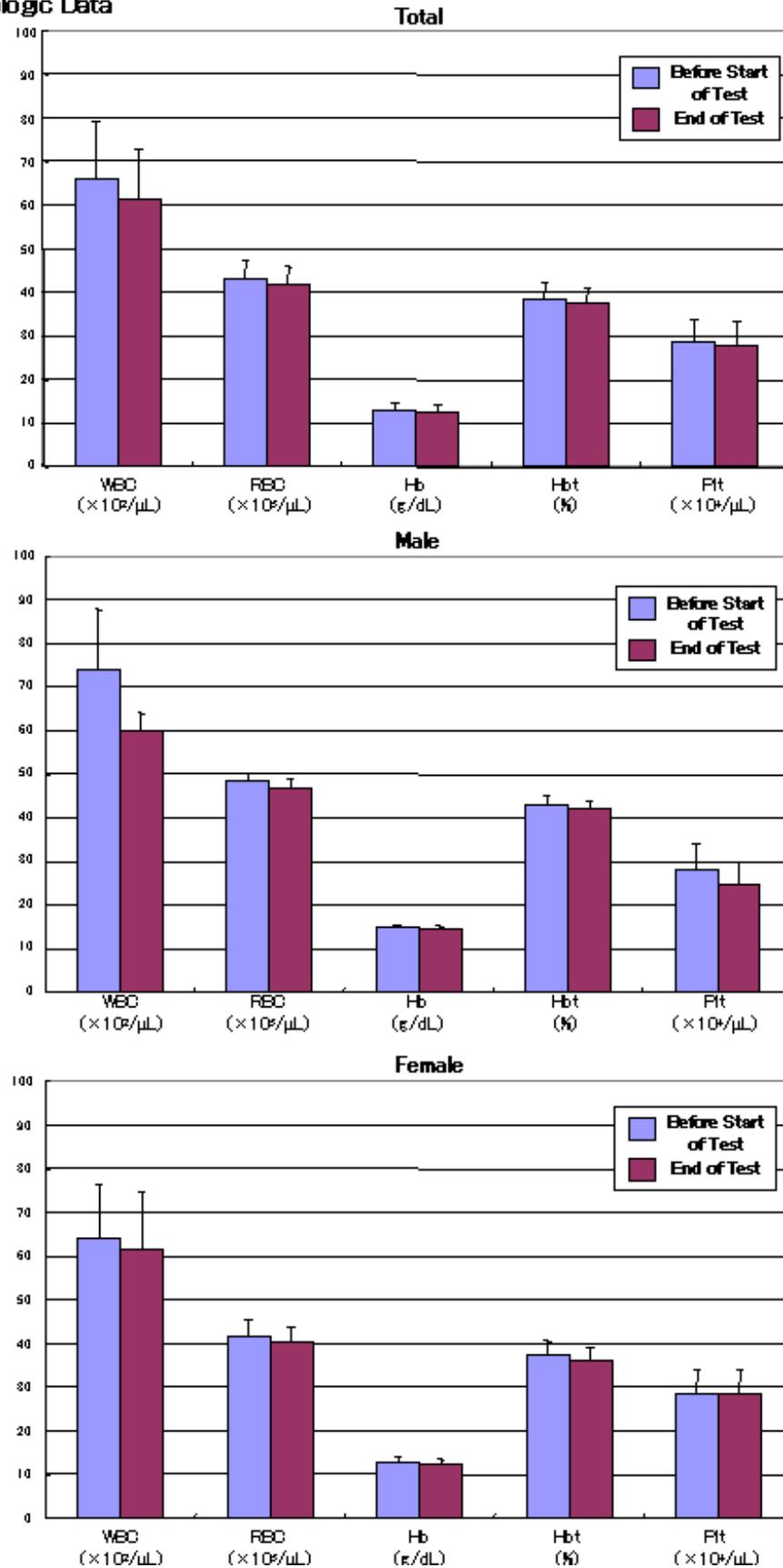


Figure 2: Effects on Hepatic Function

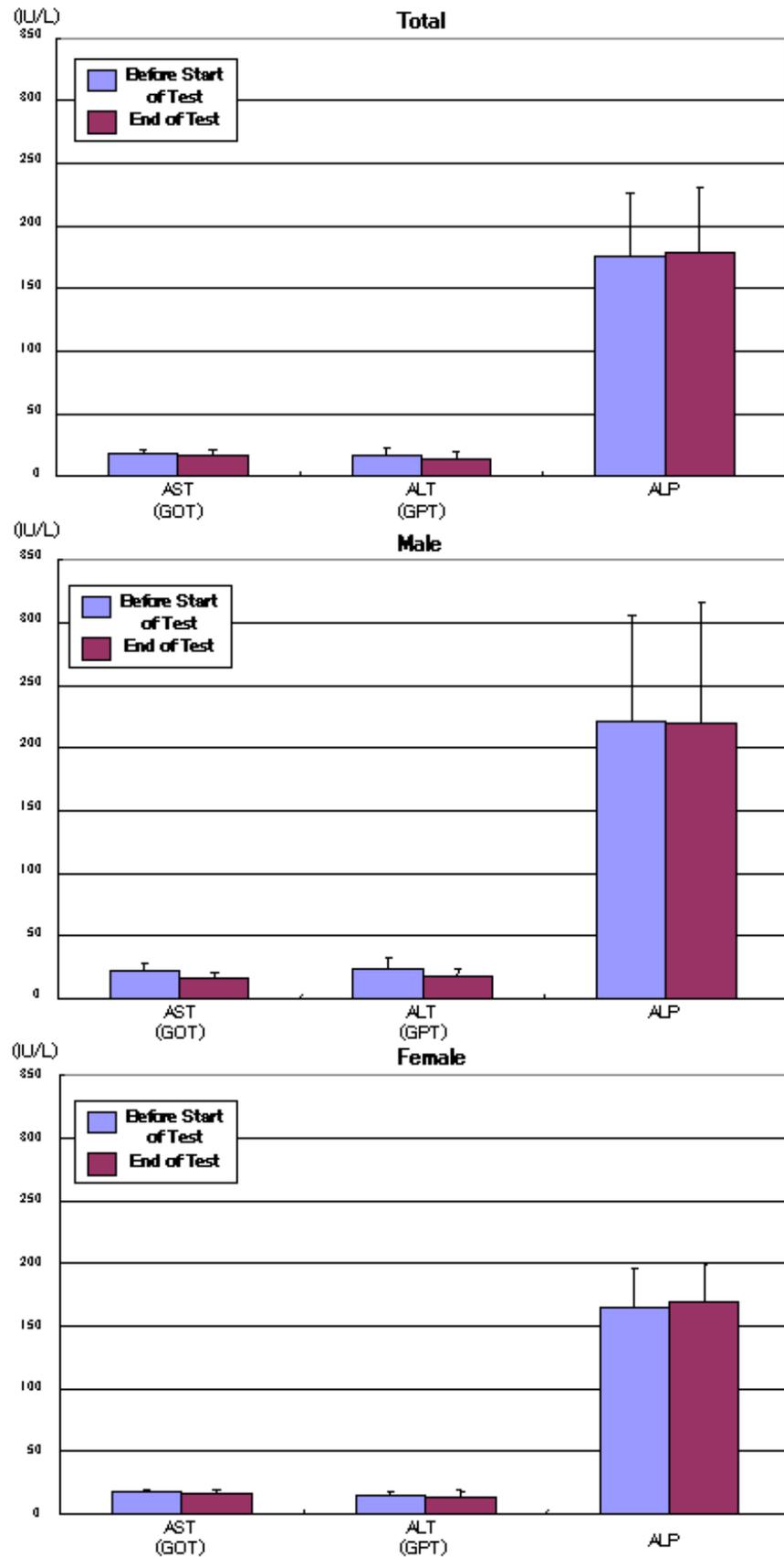


Figure 3: Effects on Lipid Metabolism

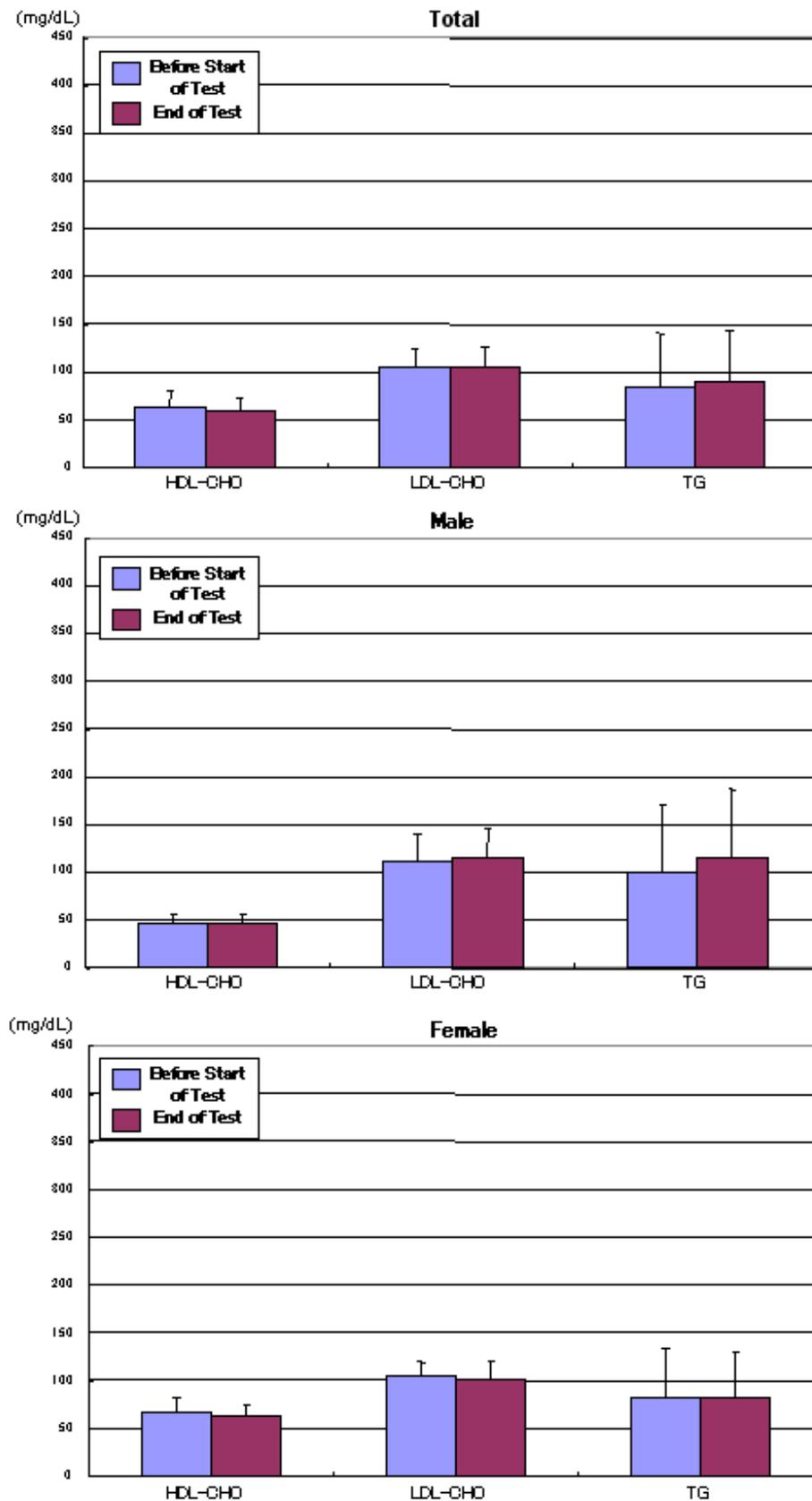


Figure 4: Effects on Renal Function

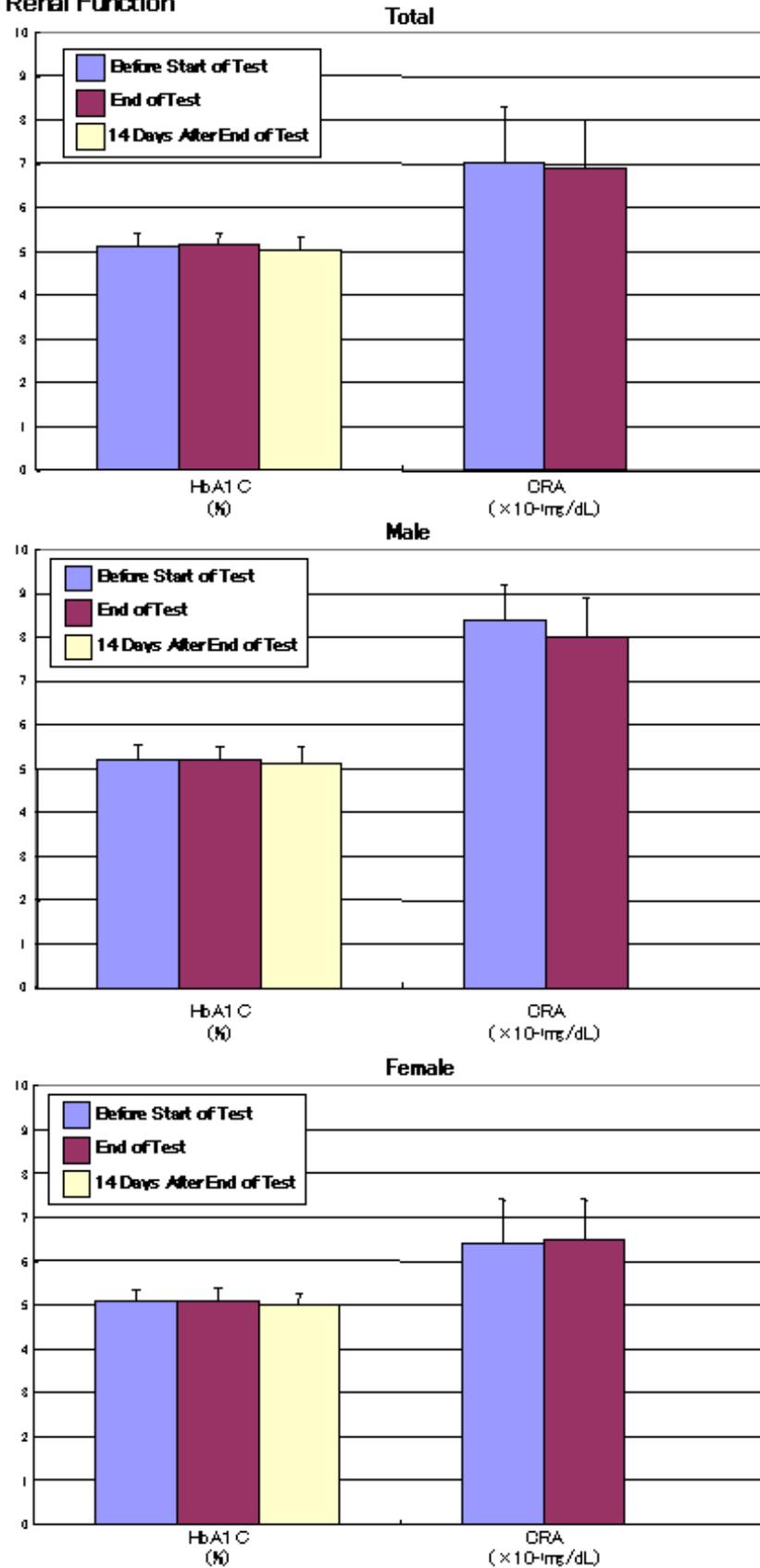


Figure 5: Lymphocyte Kinetics

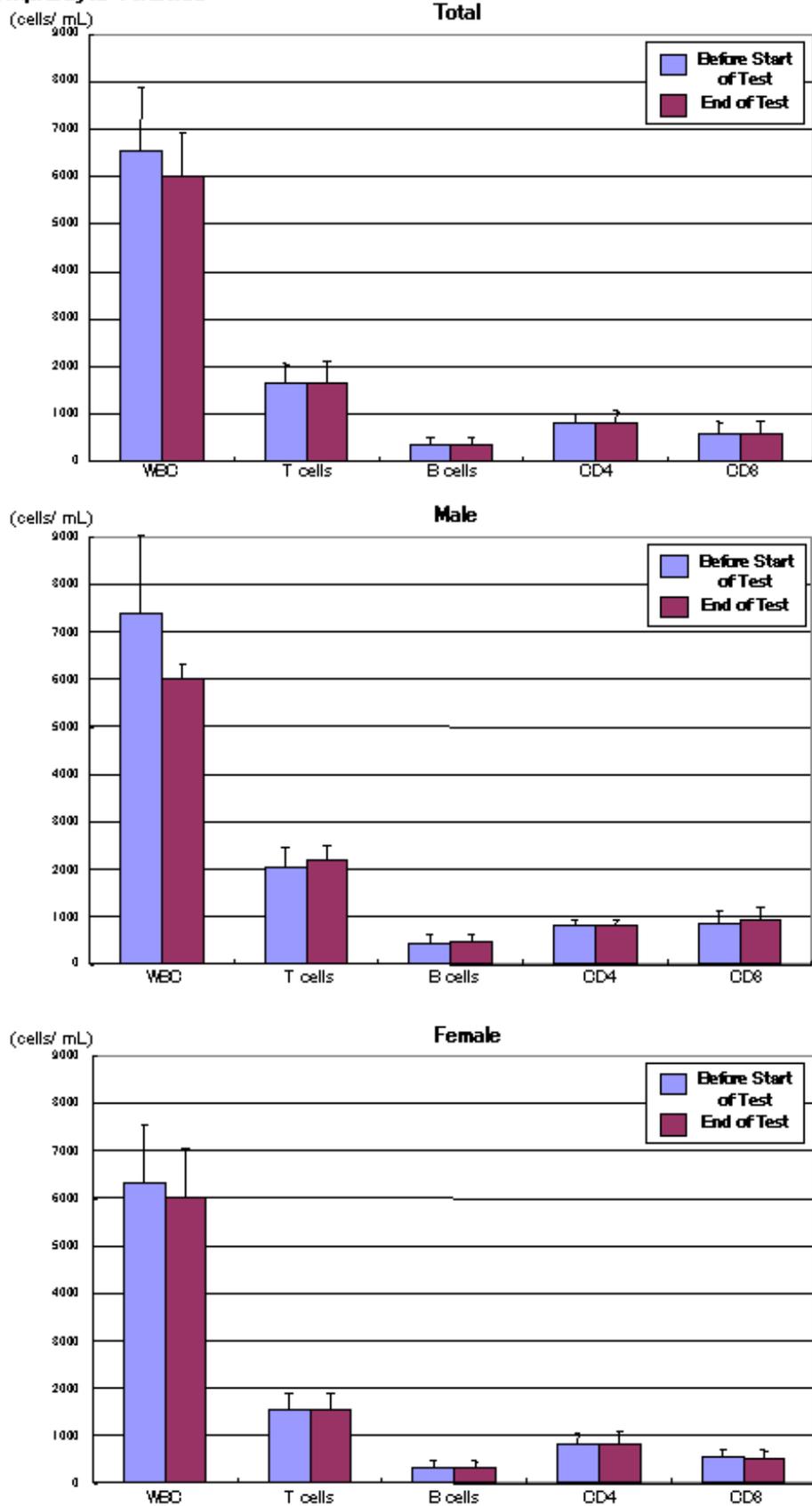


Figure 6: Effects on T-cell Function

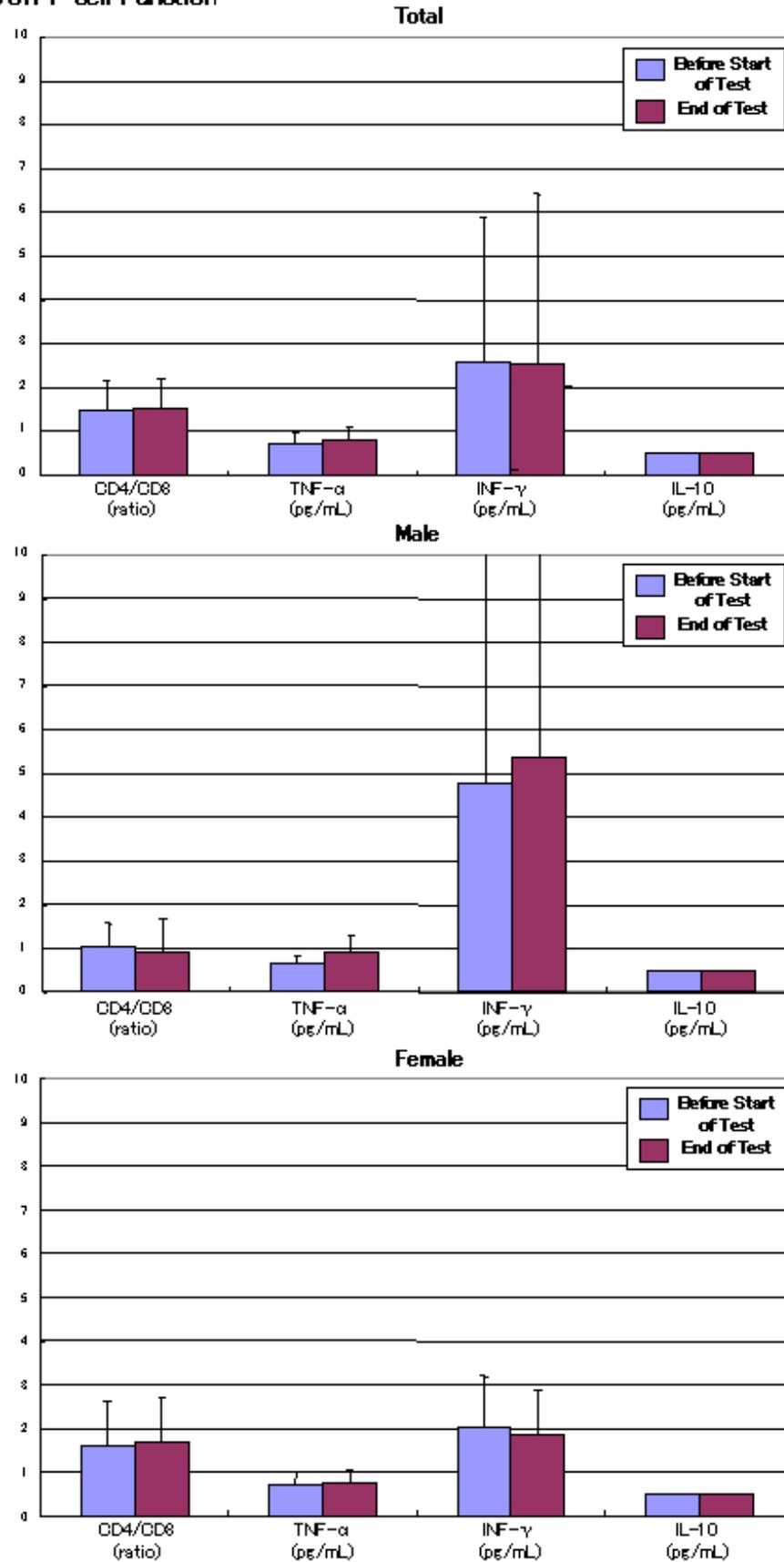


Figure 7: VAS Evaluation

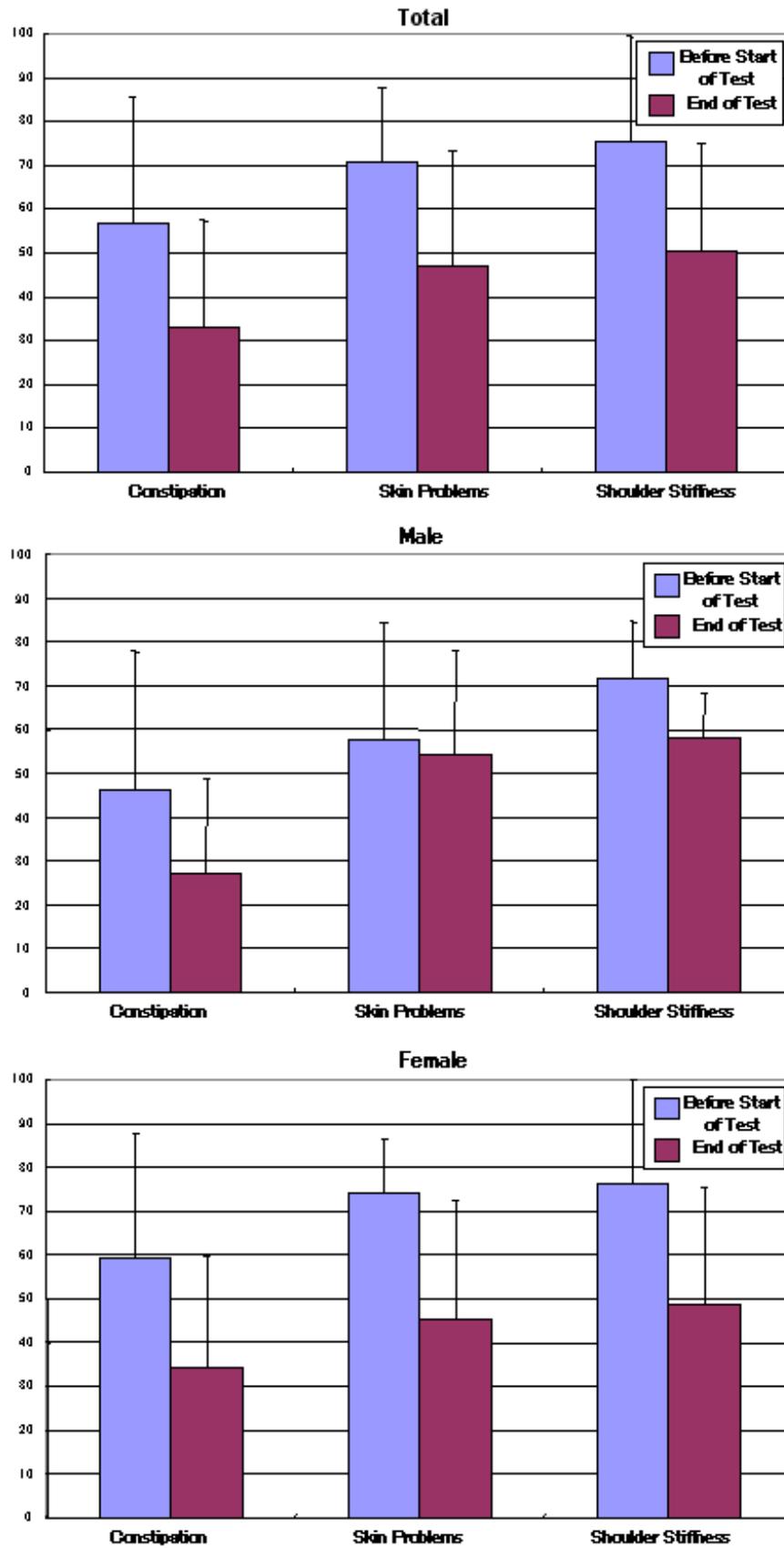


Table 1: Effects on hematology

A (Before start of test)	WBC ( $\times 10^3/\mu\text{L}$ )		RBC ( $\times 10^5/\mu\text{L}$ )		Hb (g/dL)		Hbt (%)		Plt ( $\times 10^4/\mu\text{L}$ )	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Total	66.00	$\pm 13.26$	44.21	$\pm 4.78$	13.43	$\pm 1.75$	39.43	$\pm 4.52$	27.99	$\pm 5.08$
Male	69.80	$\pm 14.63$	49.09	$\pm 2.66$	15.20	$\pm 0.88$	44.05	$\pm 2.38$	26.87	$\pm 4.46$
Female	64.10	$\pm 12.47$	41.77	$\pm 3.56$	12.54	$\pm 1.36$	37.12	$\pm 3.41$	28.55	$\pm 5.38$

B (End of test)	WBC ( $\times 10^3/\mu\text{L}$ )		RBC ( $\times 10^5/\mu\text{L}$ )		Hb (g/dL)		Hbt (%)		Plt ( $\times 10^4/\mu\text{L}$ )	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Total	62.27	$\pm 11.57$	42.84	$\pm 4.41$	13.08	$\pm 1.60$	38.38	$\pm 4.10$	27.40	$\pm 5.13$
Male	63.50	$\pm 8.75$	47.52	$\pm 1.99$	14.77	$\pm 0.78$	42.90	$\pm 1.89$	26.29	$\pm 3.52$
Female	61.65	$\pm 12.92$	40.50	$\pm 3.25$	12.24	$\pm 1.16$	36.13	$\pm 2.82$	28.46	$\pm 5.55$

Table 2: Effects on hepatic function, lipid metabolism, and renal function

A (Before start of test)	AST (GOT) (U/L)		ALT (GPT) (U/L)		ALP (U/L)		HDL-CHO (mg/dL)		LDL-CHO (mg/dL)		TG (mg/dL)		CrA (mg/dL)		HbA1C(mg/dL)	
	Average	± SD	Average	± SD	Average	± SD	Average	± SD	Average	± SD	Average	± SD	Average	± SD	Average	± SD
Total	20.03	± 6.28	21.23	± 15.59	184.17	± 52.54	60.93	± 15.12	109.77	± 24.74	119.47	± 141.85	0.70	± 0.13	5.11	± 0.30
Male	25.80	± 7.52	35.20	± 20.86	224.00	± 64.15	48.00	± 6.31	121.50	± 35.20	196.20	± 221.93	0.84	± 0.08	5.21	± 0.32
Female	17.15	± 2.68	14.25	± 3.29	164.25	± 31.81	67.40	± 14.08	103.90	± 15.44	81.10	± 52.28	0.64	± 0.10	5.07	± 0.29

B (End of test)	AST (GOT) (U/L)		ALT (GPT) (U/L)		ALP (U/L)		HDL-CHO (mg/dL)		LDL-CHO (mg/dL)		TG (mg/dL)		CrA (mg/dL)		HbA1C(mg/dL)	
	Average	± SD	Average	± SD	Average	± SD	Average	± SD	Average	± SD	Average	± SD	Average	± SD	Average	± SD
Total	19.80	± 7.03	19.73	± 12.95	186.60	± 55.51	58.27	± 12.38	108.80	± 24.70	112.17	± 106.10	0.70	± 0.12	5.14	± 0.28
Male	25.30	± 9.65	31.60	± 16.08	222.60	± 75.78	48.80	± 8.18	123.00	± 29.71	171.20	± 160.34	0.81	± 0.09	5.23	± 0.27
Female	17.05	± 2.74	13.80	± 4.71	168.60	± 30.99	63.00	± 11.47	101.70	± 18.81	82.65	± 47.46	0.65	± 0.09	5.10	± 0.28

Table 3: Effects on immune function

A (Before start of test)	WBC (cells/mL)		T cells (cells/mL)		B cells (cells/mL)		CD4 (cells/mL)		CD8 (cells/mL)		CD4/CD8 (ratio)		TNF- $\alpha$ (pg/mL)		IFN- $\gamma$ (pg/mL)		IL-10 (pg/mL)		
	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	
Total	6486.67 $\pm$ 1318.76	1669.20	387.11 $\pm$ 149.73	349.63 $\pm$ 140.73	817.53 $\pm$ 192.37	623.70 $\pm$ 235.03	1.42 $\pm$ 0.61	0.74 $\pm$ 0.30	2.40 $\pm$ 3.05	0.50 $\pm$ 0.00									
Male	6890.00 $\pm$ 1475.92	1882.00	414.14 $\pm$ 155.21	388.90 $\pm$ 155.21	813.10 $\pm$ 144.61	795.60 $\pm$ 251.58	1.06 $\pm$ 0.37	0.79 $\pm$ 0.36	3.17 $\pm$ 5.10	0.50 $\pm$ 0.00									
Female	6305.00 $\pm$ 1232.23	1562.80	334.27 $\pm$ 146.93	330.00 $\pm$ 146.93	819.75 $\pm$ 215.78	637.75 $\pm$ 176.07	1.61 $\pm$ 0.64	0.72 $\pm$ 0.28	2.01 $\pm$ 1.18	0.50 $\pm$ 0.00									
B (End of test)	WBC (cells/mL)		T cells (cells/mL)		B cells (cells/mL)		CD4 (cells/mL)		CD8 (cells/mL)		CD4/CD8 (ratio)		TNF- $\alpha$ (pg/mL)		IFN- $\gamma$ (pg/mL)		IL-10 (pg/mL)		
	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	Average $\pm$ SD	SD	
Total	6093.33 $\pm$ 943.19	1690.80	432.44 $\pm$ 140.52	356.50 $\pm$ 140.52	844.57 $\pm$ 242.82	637.90 $\pm$ 255.64	1.44 $\pm$ 0.64	0.79 $\pm$ 0.29	2.38 $\pm$ 3.54	0.50 $\pm$ 0.00									
Male	6260.00 $\pm$ 757.48	1996.80	408.47 $\pm$ 147.80	413.60 $\pm$ 147.80	866.20 $\pm$ 214.79	879.10 $\pm$ 217.09	0.99 $\pm$ 0.34	0.87 $\pm$ 0.31	3.46 $\pm$ 6.02	0.50 $\pm$ 0.00									
Female	6010.00 $\pm$ 1031.55	1537.80	363.98 $\pm$ 131.89	330.95 $\pm$ 131.89	833.75 $\pm$ 260.33	517.30 $\pm$ 177.47	1.67 $\pm$ 0.63	0.76 $\pm$ 0.28	1.84 $\pm$ 1.03	0.50 $\pm$ 0.00									

Table 4: Alteration of intestinal flora

Before start of test				End of test			
Clostridium	halnewyi	8	0.3%	Clostridium	raiosum	30	1.2%
	lituseburense	12	0.4%		spiriforme	28	1.1%
	bolleae	66	2.5%		neville	12	0.5%
	spiriforme	31	1.2%				
	orbiscindense	13	0.5%				
Bacteroides	ovatus	130	4.9%	Bacteroides	fragilis	70	2.8%
	vulgatus	7	0.3%		fragilis	23	0.9%
	stercoris	51	1.9%		vulgatus	56	2.2%
		78	2.9%		stercoris	87	3.4%
					caecae	23	0.9%
Streptococcus		136	5.1%	Streptococcus	salivarius	189	7.5%
	mecononicus	23	0.9%		luteitensis	21	0.8%
Ruminococcus	obeum	144	5.4%	Ruminococcus	obeum	73	2.9%
	schinkii	11	0.4%		forques	81	3.2%
	gnavus	9	0.3%		gnavus	9	0.4%
	forques	32	1.2%			22	0.9%
	lactaris	9	0.3%				
	hydrogenotrophicus	11	0.4%				
	luti	10	0.4%				
		226	8.5%			112	4.4%
Eubacterium	rectale	34	1.3%	Eubacterium	rectale	25	1.0%
	biforme	25	0.9%		biforme	16	0.6%
Faecalibacterium		59	2.2%	Faecalibacterium		41	1.6%
	prausnitzii	176	6.6%		prausnitzii	150	5.9%
	oulorum	170	6.4%		oulorum	207	8.2%
Prevotella	invisus	15	0.6%	Prevotella			
Anaerostipes	caecae	10	0.4%	Anaerostipes	caecae	25	1.0%
				Turcibacter	sanguinis	17	0.7%
				Megamonas	hypermegale	10	0.4%
total clone		2667		total clone		2525	

Chart 1

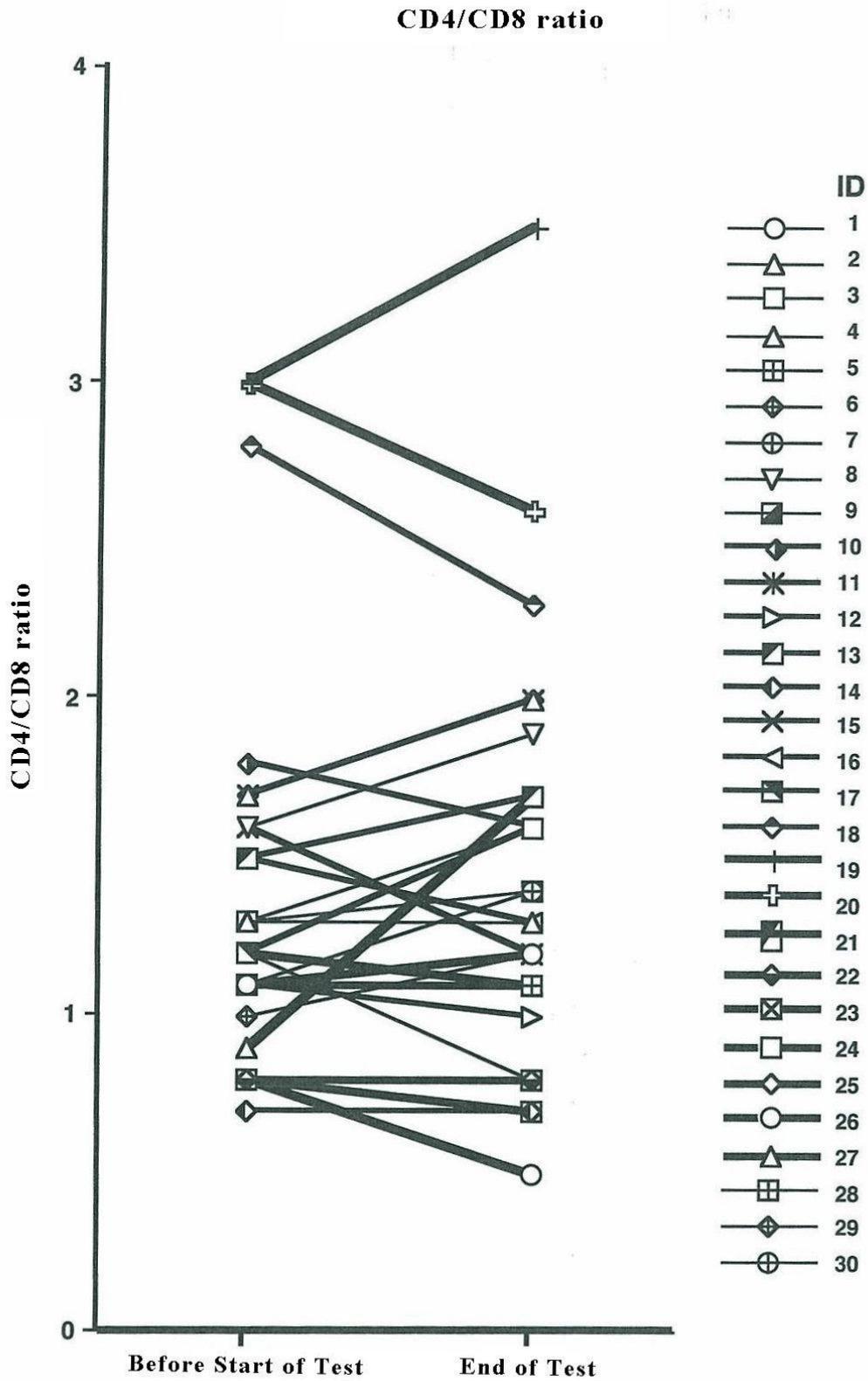


Chart 2

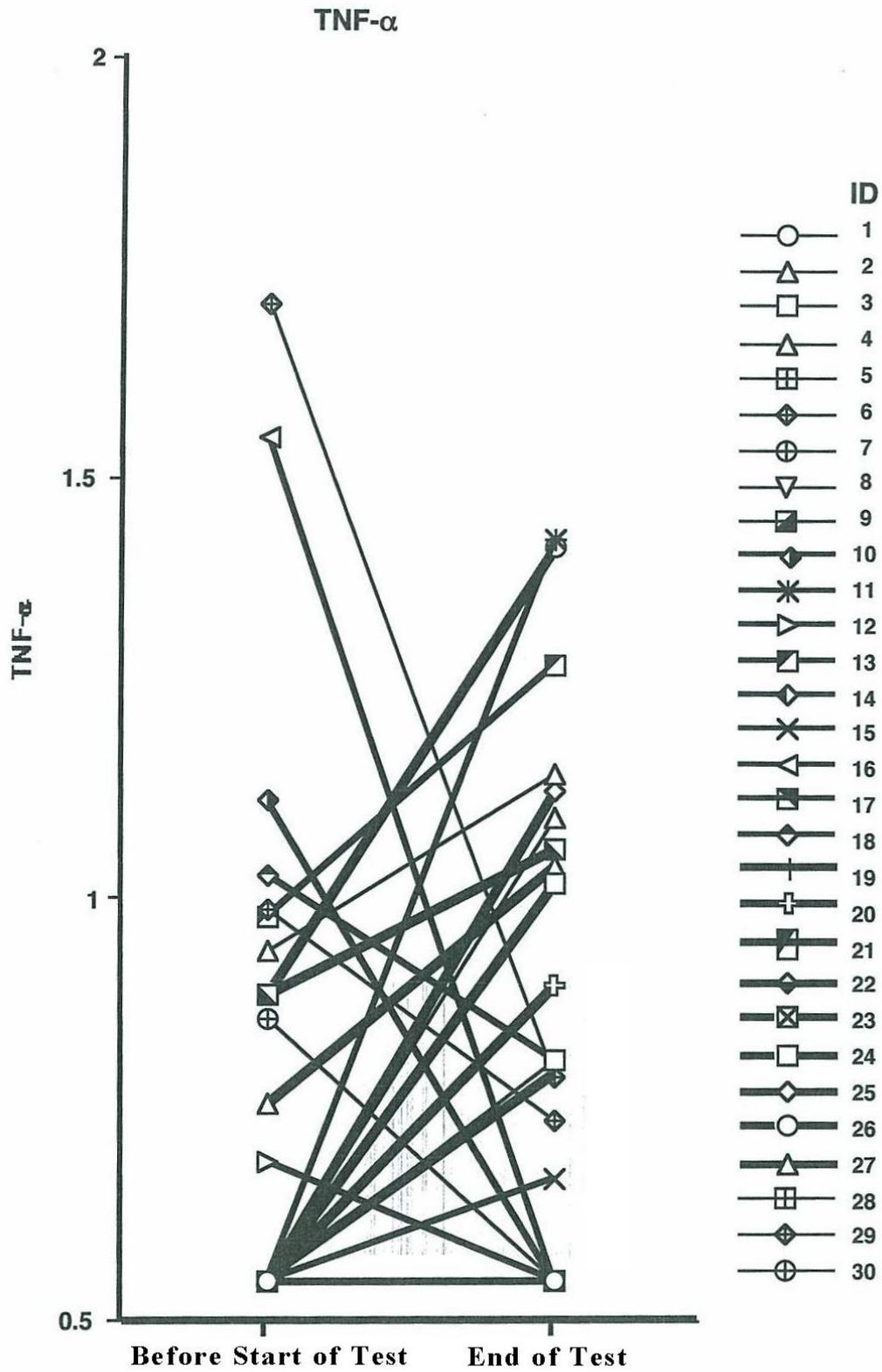
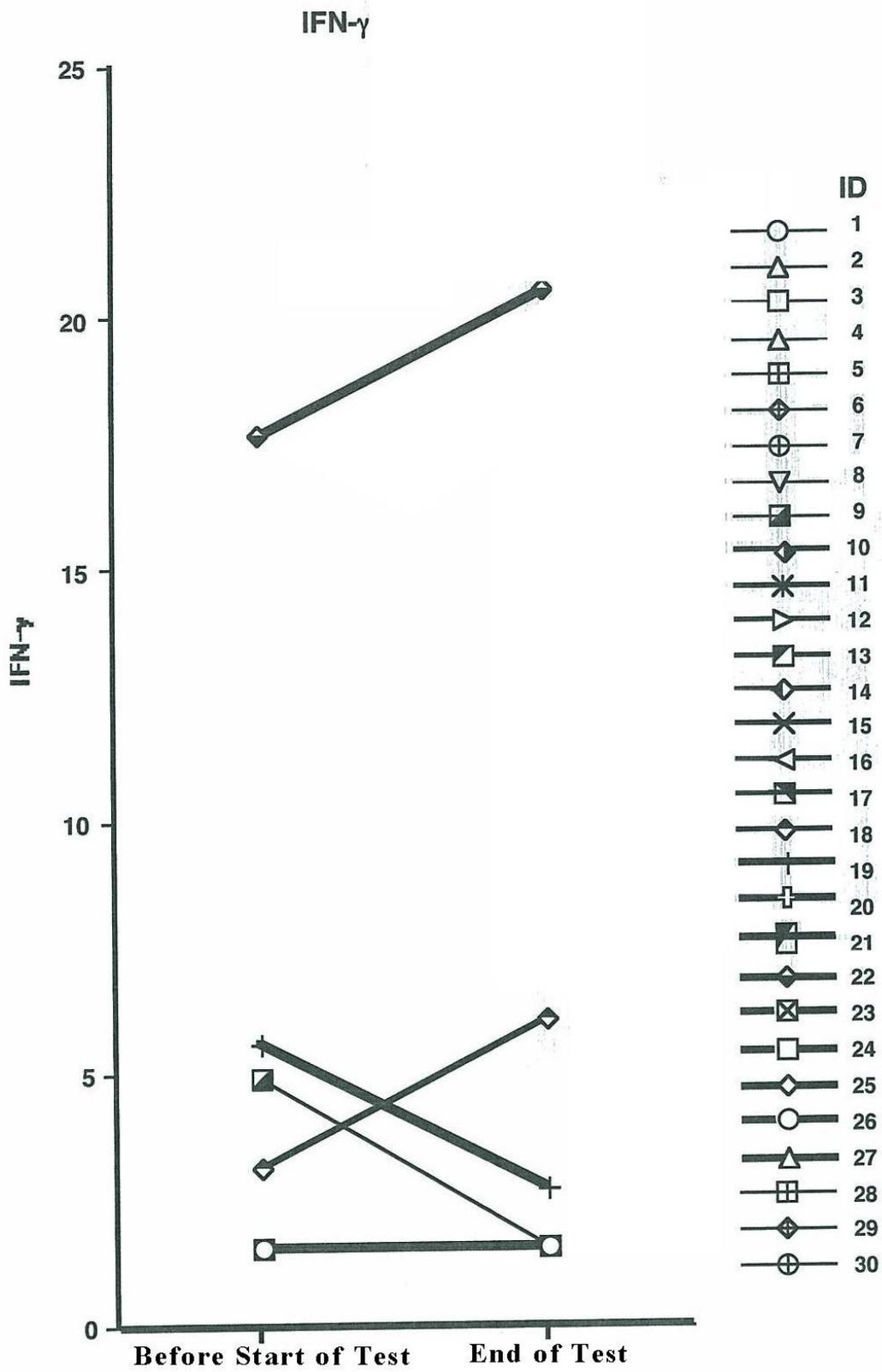
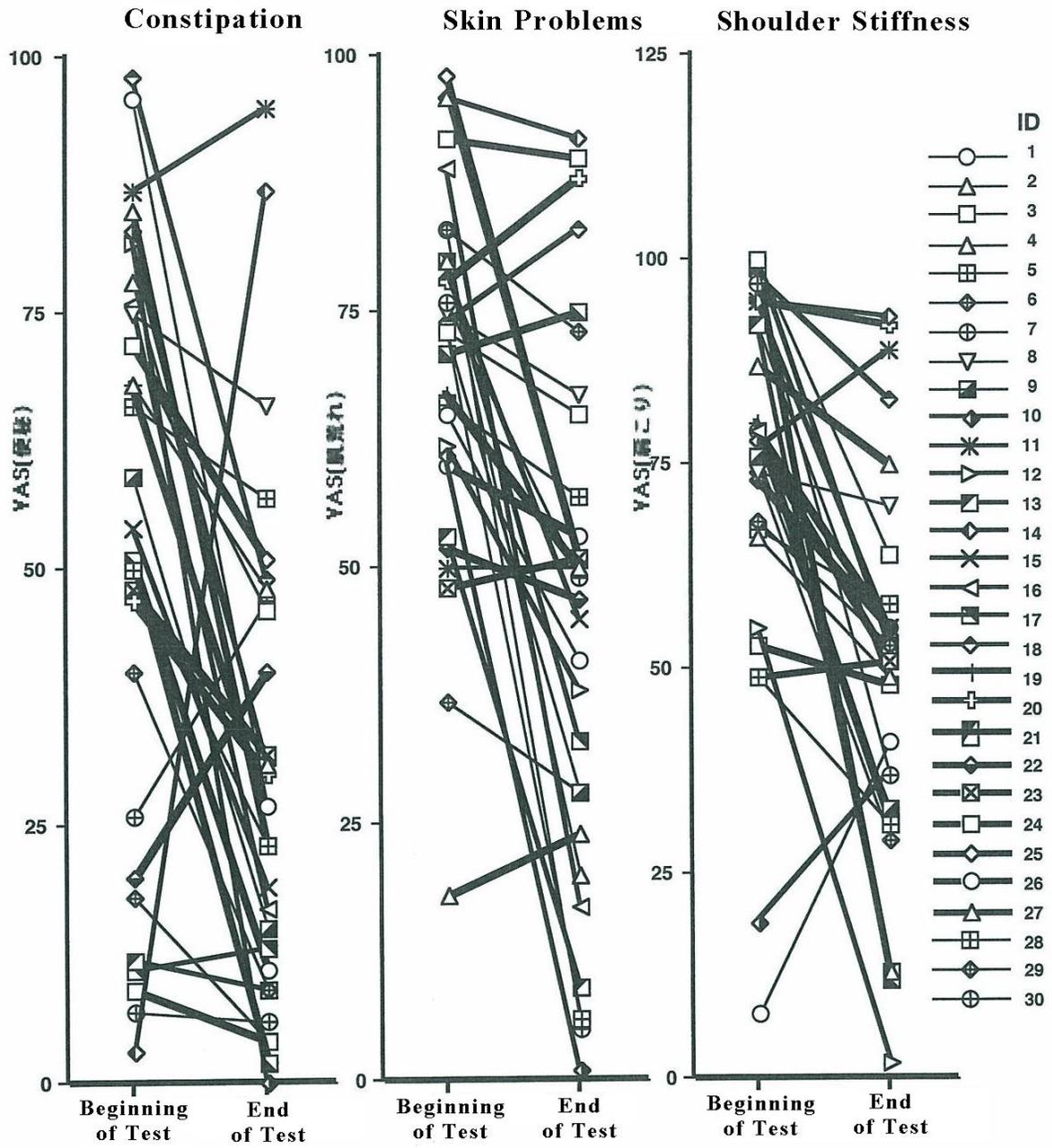


Chart 3



**Chart 4**



Intestinal bacterial flora count and destruction rate before oral administration of preB®

Sample #	Weight (g)	Color	Hardness	Quantity of food residue	Flora count before test (colonies/g)	Flora count after test	Destruction rate (%)
1	0.100	Brown	Hard	Small	$1.6 \times 10^{10}$	$5.4 \times 10^{10}$	71.4
2	0.100	Ocher	Soft	Small	$6.8 \times 10^{10}$	$9.4 \times 10^9$	86.1
3	0.100	Ocher	Soft	Small	$1.6 \times 10^{10}$	$2.2 \times 10^6$	98.6
4	0.100	Dark brown	Hard	Small	$5.2 \times 10^{10}$	$7.1 \times 10^9$	86.5
5	0.100	Ocher	Soft (partly sludged)	Small	$4.5 \times 10^{10}$	$5.6 \times 10^9$	87.6
6	0.100	Brown	Soft	Small	$5.6 \times 10^{10}$	$4.8 \times 10^9$	91.5
7	0.100	Brown	Average	Small	$5.2 \times 10^{10}$	$2.4 \times 10^9$	95.4
8	0.100	Brown	Soft (partly sludged)	Small	$6.9 \times 10^{10}$	$7.6 \times 10^9$	88.9
9	0.100	Brown	Soft	Small	$4.3 \times 10^{10}$	$2.1 \times 10^9$	95.1
10	0.100	Brown	Soft	Small	$3.5 \times 10^{10}$	$4.0 \times 10^9$	88.6
11	0.101	Brown	Hard	Small	$6.7 \times 10^{10}$	$7.4 \times 10^9$	88.8
12	0.099	Brown	Soft	Small	$1.0 \times 10^{11}$	$8.7 \times 10^9$	91.3
13	0.100	Brown	Soft	Small	$4.9 \times 10^{10}$	$2.8 \times 10^9$	94.3
14	0.100	Ocher	Very soft	Average	$8.9 \times 10^{10}$	$1.3 \times 10^9$	85.4
15	0.097	Dark brown	Hard	Small	$8.1 \times 10^{10}$	$1.1 \times 10^{10}$	86.4
16	0.097	Brown	Hard	Small	$1.0 \times 10^{11}$	$1.1 \times 10^{10}$	88.8
17	0.107	Brown	Very soft	Average	$8.5 \times 10^{10}$	$4.0 \times 10^9$	95.3
18	0.101	Brown	Soft	Small	$4.5 \times 10^{10}$	$6.0 \times 10^9$	86.7
19	0.099	Brown	Hard	Average	$9.4 \times 10^{10}$	$9.1 \times 10^9$	90.3
20	0.101	Brown	Soft	Small	$2.8 \times 10^{10}$	$5.0 \times 10^9$	81.7
21	0.035	Ocher	Soft (partly sludged)	Small	$1.0 \times 10^{10}$	$8.5 \times 10^8$	91.7
22	0.103	Brown	Soft	Average	$3.1 \times 10^{10}$	$6.0 \times 10^6$	98.1
23	0.102	Ocher	Hard	Small	$3.1 \times 10^{10}$	$7.4 \times 10^7$	99.2
24	0.098	Brown	Soft	Small	$6.2 \times 10^{10}$	$1.1 \times 10^9$	94.6
25	0.101	Ocher	Average	Small	$7.7 \times 10^{10}$	$7.4 \times 10^7$	99.7
26	0.091	Dark brown	Average	Small	$7.7 \times 10^{10}$	$1.5 \times 10^9$	94.2
27	0.098	Brown	Soft	Small	$6.8 \times 10^{10}$	$6.7 \times 10^9$	90.2
28	0.099	Ocher	Soft (partly sludged)	Small	$6.2 \times 10^9$	$2.5 \times 10^7$	98.8
29	0.098	Ocher	Sludged	Average	$9.9 \times 10^{10}$	$1.2 \times 10^8$	99.6
30	0.101	Brown	Soft	Small	$7.1 \times 10^{10}$	$3.5 \times 10^8$	98.5

Intestinal bacterial flora analysis before oral administration of preB

no. 1	Superkingdom	Num. phylum	Num. class	Num. order	Num. family	Num. genus	Num. species	Num. Blast Max%	Blast Min%
94	Proteobacteria	1 Gammaproteobacteria	1 Enterobacteriales	1 Escherichia coli	1 Escherichia coli	1	100	99	100
94	Firmicutes	72 "Erysipelotrichi"	6 "Erysipelotrichales"	6 Erysipelotrichaceae	6 Erysipelotrichaceae Incertae Sedis	6 Clostridium innocuum	3	99	99
		Bacilli	35 Lactobacillales	35 Streptococcaceae	32 Streptococcus	Clostridium sporforme	2	100	91
		Clostridia	31 Clostridiales	31 Clostridiaceae	1 Lactobacillus	Streptococcus parasanguinis	5	99	99
					2 Enterococcus	Streptococcus macedonicus	23	100	98
					20 Clostridium	Streptococcus sinensis	1	99	99
						1 Lactobacillus paracasei	1	99	99
						2 Enterococcus pseudovivium	2	88	88
						16 Clostridium leptum	2	99	95
						Clostridium sporosphaeroides	1	93	93
						Clostridium sphenoides	1	95	95
						Clostridium xylinolyticum	1	92	92
						Clostridium orthoferdens	1	94	94
						Clostridium hylemonae	1	99	99
						Clostridium hahwayi	1	99	99
						Clostridium boltae	8	96	94
						1 Sporobacter terrificus	1	94	94
						3 Faecalibacterium prausnitzii	3	98	92
						8 Ruminococcus gnavus	2	99	94
						Ruminococcus sobrum	5	97	96
						Ruminococcus sibiriacus	1	97	97
						1 Anaerostipes codonutans	1	99	99
						1 Anaerostipes halii	1	99	98
						1 Anaerostipes codonutans	1	94	94
						18 Bacteroides fragilis	2	100	99
						Bacteroides distasonis	2	98	98
						Bacteroides vulgatus	1	88	86
						Bacteroides distasonis	1	88	86
						Bacteroides ovatus	7	98	97
						Bacteroides merdae	1	99	99
						Bacteroides stercoris	5	99	97
						1 Alistipes putredinis	1	97	97
						1 Collinsella intestinalis	1	98	98
						1 Actinomyces naestlundii	1	98	98
94	Actinobacteria	2 Actinobacteria (class)	2 Coriobacteriales	1 Coriobacteriaceae	Alistipes	1	94	94	98
					1 Actinomycetales	1 Collinsella	1	98	98
						1 Actinomycetales	1	98	98
94	Actinobacteria	2 Actinobacteria (class)	2 Coriobacteriales	1 Coriobacteriaceae	Alistipes	1	94	94	98
					1 Actinomycetales	1 Collinsella	1	98	98
						1 Actinomycetales	1	98	98
no. 2	no. 2	no. 2	no. 2	no. 2	no. 2	no. 2	no. 2	no. 2	no. 2
Superkingdom	Num. phylum	Num. class	Num. order	Num. family	Num. genus	Num. species	Num. Blast Max%	Blast Min%	
Bacteria	84 Proteobacteria	3 Betaproteobacteria	3 Burkholderiales	3 Burkholderiaceae	3 Ralstonia	3 Ralstonia insidiosa	3	88	86
	Firmicutes	55 "Erysipelotrichi"	2 "Erysipelotrichales"	2 Erysipelotrichaceae	2 Erysipelotrichaceae Incertae Sedis	2 Clostridium innocuum	1	99	99
		Bacilli	5 Lactobacillales	5 Streptococcaceae	4 Streptococcus	Clostridium ramosum	1	99	99
		Clostridia	48 Clostridiales	48 Clostridiaceae	1 Lactobacillus	4 Streptococcus salivarius	4	99	98
					48 Acidimicrobaceae	1 Phascolarctobacterium faecium	1	99	99
					15 Clostridium	1 Phascolarctobacterium faecium	1	100	100
						15 Clostridium	1	90	90
						Clostridium xylinolyticum	4	95	95
						Clostridium boltae	6	95	95
						Dorea	1	99	99
						1 Dorea longicatena	1	99	99
						3 Faecalibacterium prausnitzii	3	98	96
						6 Roseburia intestinalis	6	96	96
						27 Roseburia	2	100	100
						Ruminococcus gnavus	2	98	98
						Ruminococcus bromii	3	98	98
						Ruminococcus obeum	11	97	96
						Ruminococcus hydrogenotrophicus	3	97	92
						Ruminococcus sobirkii	1	92	92
						1 Lachnospira pectinohizcha	1	98	98
						5 Eubacterium raminus	4	97	97
						Eubacterium ventriosum	1	99	99
						25 Bacteroides vulgatus	13	99	98
						Bacteroides distasonis	1	98	98
						Bacteroides ovatus	1	99	98
						Bacteroides stercoris	7	99	98
						Bacteroides caccae	3	99	95
84	Unclassified bacteria	1 Unclassified bacteria	1 Unclassified bacteria	1 Unclassified bacteria	1 Unclassified bacteria	1 Uncultured bacterium clone R103M 68	1	84	95









no. 11																	
superkingdom	Num	phylum	Num	class	Num	order	Num	family	Num	genus	Num	species	Num	Blast	Max%	Blast	Min%
Bacteria	91	Firmicutes	53	"Erysipelotrichi"	2	"Erysipelotrichales"	2	Erysipelotrichaceae	20	Erysipelotrichaceae	Incertae Sedis	2	Clostridium sporiforme	5	94	95	94
				Clostridia	51	Clostridiales	51	Clostridiaceae		Dorea	1	Dorea longicatena	1	99	99	95	95
								Lachnospiraceae		Faecalibacterium	14	Faecalibacterium prausnitzii	14	98	98	92	92
										Ruminococcus	25	Ruminococcus znavus	20	100	100	96	96
										Ruminococcus		Ruminococcus torques	2	99	99	86	86
										Ruminococcus		Ruminococcus obeum	1	99	99	86	86
										Ruminococcus		Ruminococcus hydrogenophilicus	5	97	97	97	97
										Ruminococcus		Ruminococcus luti	10	98	98	97	97
										Lachnospira		Lachnospira pectusobliqua	1	97	97	97	97
										Anaerofilum		Anaerofilum parvosporans	1	87	87	96	96
										Anaerostipes		Anaerostipes caccae	3	96	96	96	96
										Eubacterium		Eubacterium eligens	6	92	92	92	92
										Eubacterium		Eubacterium hallii	5	92	92	92	92
										Bacteroides		Bacteroides vulgatus	14	99	99	99	99
										Bacteroides		Bacteroides distasonis	1	86	86	86	86
										Bacteroides		Bacteroides merdae	2	99	99	99	99
										Bacteroides		Bacteroides stercoris	7	99	99	99	99
										Bacteroides		Bacteroides caccae	4	99	99	98	98
										Bacteroides		Bacteroides acidifaciens	3	98	98	95	95
										Alistipes		Alistipes putredinis	3	97	97	97	97
										Collinsella		Collinsella aerofaciens	2	99	99	99	99
										Collinsella		Collinsella aerofaciens	2	99	99	95	95
										Uncultured bacterium		Uncultured bacterium adhufac12.25	4	99	99	95	95
										Uncultured bacterium		Uncultured bacterium dhrom323-86-26	1	97	97	97	97
													91	91	91	91	91
no. 12																	
superkingdom	Num	phylum	Num	class	Num	order	Num	family	Num	genus	Num	species	Num	Blast	Max%	Blast	Min%
Bacteria	84	Firmicutes	52	Clostridia	52	Clostridiales	52	Acidaminococcaceae	38	Megasphaera	1	Megasphaera micronuciformis	7	92	92	92	92
								Clostridiaceae		Clostridium		Clostridium nexile	1	97	97	97	97
										Clostridium		Clostridium colinum	1	95	95	95	95
										Clostridium		Clostridium viride	1	90	90	90	90
										Clostridium		Clostridium hathewayi	1	96	96	96	96
										Clostridium		Clostridium botlhaeae	3	95	95	95	95
										Sporobacter		Sporobacter termidis	1	91	91	91	91
										Dorea		Dorea longicatena	1	99	99	99	99
										Faecalibacterium		Faecalibacterium prausnitzii	29	98	98	96	96
										Ruminococcus		Ruminococcus bromii	9	92	92	92	92
										Ruminococcus		Ruminococcus obeum	2	99	99	99	99
										Ruminococcus		Ruminococcus hydrogenophilicus	2	97	97	96	96
										Ruminococcus		Ruminococcus luti	3	98	98	98	98
										Anaerostipes		Anaerostipes caccae	3	96	96	96	96
										Eubacterium		Eubacterium desmolans	1	96	96	96	96
										Bacteroides		Bacteroides vulgatus	21	98	98	95	95
										Bacteroides		Bacteroides distasonis	0	86	86	86	86
										Bacteroides		Bacteroides ovatus	1	86	86	80	80
										Bacteroides		Bacteroides merdae	2	99	99	99	99
										Bacteroides		Bacteroides stercoris	1	99	99	99	99
										Bacteroides		Bacteroides caccae	2	99	99	88	88
										Alistipes		Alistipes putredinis	1	97	97	97	97
										Dysgonomonas		Dysgonomonas mossii	1	84	84	84	84
										Collinsella		Collinsella aerofaciens	6	99	99	96	96
										Bifidobacterium		Bifidobacterium adolescentis	2	97	97	95	95
										Uncultured bacterium		Uncultured bacterium adhufac12.25	1	95	95	95	95
										Uncultured bacterium		Uncultured bacterium adhufac84	1	95	95	95	95
													84	84	84	84	84



no.	superkingdom	num	phylum	num	class	num	order	num	family	num	genus	num	species	num	blast	max%	blast	min%	
83	Proteobacteria	5	Gammaproteobacteria	5	Erythrobacterales	5	Erythrobacterales	5	Erythrobacteriaceae	5	Escherichia	5	Escherichia coli	5	100	98	98	98	
83	Firmicutes	66	"Erysipelotrichi"	10	"Erysipelotrichales"	10	Erysipelotrichales	10	Erysipelotrichaceae	10	Erysipelotrichaceae	10	Streptococcus pneumoniae	10	100	99	99	99	
			Bacilli	4	Lactobacillales	4	Streptococaceae	4	Streptococaceae	1	Streptococcus	1	Clostridium ramosum	1	99	99	99	99	
			Clostridia	52	Clostridiales	52	Clostridiaceae	52	Clostridiaceae	3	Streptococcus salivarius	3	Clostridium spiriforme	3	100	98	90	100	
										3	Lactobacillus	3	Lactobacillus orispathus	3	89	89	89	89	
										15	Clostridium	15	Clostridium cellulosilyticum	1	89	89	89	89	
													Clostridium leptum	1	97	97	97	97	
													Clostridium flussaburense	4	97	95	95	95	
													Clostridium celerecreans	1	96	96	96	96	
													Clostridium irregulare	1	98	98	98	98	
													Clostridium viride	1	91	91	91	91	
													Clostridium indolis	1	95	95	95	95	
													Clostridium orbiscindens	1	94	94	94	94	
													Clostridium hyemense	1	93	93	93	93	
													Faecalibacterium prausnitzii	3	97	96	96	96	
													Ruminococcus productus	37	100	100	100	100	
													Ruminococcus granus	1	96	96	96	96	
													Ruminococcus torques	1	98	98	98	98	
													Ruminococcus bromii	16	98	98	98	98	
													Ruminococcus lactaris	7	96	96	96	96	
													Ruminococcus hydrogenotrophicus	1	97	97	97	97	
													Ruminococcus luti	1	96	96	96	96	
													Ruminococcus soehinki	1	97	97	97	97	
													Bacteroides vulgatus	3	97	97	97	97	
													Alistipes putredinis	1	90	89	89	89	
													Eggerthella lenta	2	97	97	97	97	
													Collinsella aerofaciens	5	99	97	97	97	
													Bifidobacterium adolescentis	2	97	97	97	97	
													Uncultured bacterium clone p-1561-05	1	97	97	97	97	
													Unclassified bacteria	83	83	83	83	83	
83	Unclassified bacteria	1	Unclassified bacteria	83	Unclassified bacteria	83	Unclassified bacteria	83	Unclassified bacteria	83	Unclassified bacteria	83	Unclassified bacteria	83	83	83	83	83	83
no. 16	superkingdom	num	phylum	num	class	num	order	num	family	num	genus	num	species	num	blast	max%	blast	min%	
88	Bacteria	88	Firmicutes	65	"Erysipelotrichi"	5	"Erysipelotrichales"	5	Erysipelotrichaceae	5	Erysipelotrichaceae	5	Incertae Sedis	5	99	94	94	99	
			Bacilli	4	Lactobacillales	4	Streptococaceae	4	Streptococaceae	4	Streptococcus	4	Eubacterium dolichum	2	94	94	94	94	
			Clostridia	56	Clostridiales	56	Acidiminococaceae	56	Acidiminococaceae	1	Dialister	1	Eubacterium tortuosum	1	95	95	95	95	
													Streptococcus salivarius	4	100	100	100	100	
													Dialister invisus	1	100	100	100	100	
													Clostridium leptum	11	97	97	88	97	
													Clostridium sporosphaeroides	2	94	94	93	93	
													Clostridium nexile	1	97	97	97	97	
													Clostridium methylpentosum	1	91	91	91	91	
													Clostridium hyemense	2	96	96	95	95	
													Faecalibacterium prausnitzii	4	96	88	88	88	
													Roseburia intestinalis	3	96	96	96	96	
													Ruminococcus productus	36	99	99	99	99	
													Ruminococcus granus	1	99	99	99	99	
													Ruminococcus torques	1	94	94	94	94	
													Ruminococcus bromii	7	98	89	89	89	
													Ruminococcus obeum	19	96	96	96	96	
													Ruminococcus lactaris	1	95	95	95	95	
													Ruminococcus hydrogenotrophicus	1	97	97	95	95	
													Ruminococcus soehinki	1	97	97	97	97	
													Eubacterium hallii	1	96	96	96	96	
													Bacteroides vulgatus	15	98	98	98	98	
													Bacteroides stercoris	3	96	96	96	96	
													Bacteroides caccae	7	97	97	95	95	
													Alistipes putredinis	6	94	94	94	94	
													Collinsella intestinalis	2	92	92	92	92	
88	Actinobacteria	2	Actinobacteria (class)	2	Coriobacteriales	2	Coriobacteriaceae	2	Coriobacteriaceae	2	Alicispa	2	Collinsella	2	88	88	88	88	

no. 17	superkingdom	Num. phylum	Num. class	Num. order	Num. family	Num. genus	Num. species	Num. Blast	Max% Blast	Min%
89	Bacteria	89	37	1	1	1	1	99	99	99
			Bacilli	Bacillales	Turbidibacteraceae	Turbidibacter	Turbidibacter sanguinis	1	94	94
			Clostridia	Clostridiales	Acidimicrobiaceae	Phascolarctobacterium	Phascolarctobacterium faecium	1	94	94
					Clostridiaceae	Clostridium	Clostridium sphenoides	7	94	94
							Clostridium duperium	2	99	96
							Clostridium obscurum	2	96	95
							Clostridium algaivivum	1	83	83
							Clostridium hathewayi	1	89	89
							Faecalibacterium prausnitzii	12	98	96
							Ruminococcus flavifaciens	10	93	93
							Ruminococcus torques	1	96	96
							Ruminococcus obeum	2	96	96
							Ruminococcus lactaris	1	97	97
							Ruminococcus lactis	5	98	98
							Ruminococcus luti	1	97	97
							Anaerostipes caecae	1	96	96
							Anaerostipes hallii	1	98	98
							Eubacterium caecae	4	99	99
							Eubacterium hallii	2	99	97
							Eubacterium rusciale	1	96	96
							Eubacterium rumintantum	1	91	91
							Anaerovorax odorifluans	1	91	91
							Anaerovorax vulgatus	15	99	99
							Bacteroides merdae	2	99	99
							Bacteroides merdae	2	99	99
							Bacteroides stercoris	9	88	88
							Bacteroides caecae	3	89	89
							Bacteroides stercoris	9	89	90
							Prevotella bivia	29	90	90
							Prevotella corporis	1	91	91
							Prevotella outorum	1	87	87
							Shackia exigua	1	90	90
							Collinsella aerofaciens	2	99	98
							Uncultured bacterium adhufecf1_25	1	95	95
							Uncultured bacterium	85	85	85
no. 18	Actinobacteria	85	1	1	1	1	1	99	99	99
			Unclassified bacteria	Unclassified bacteria	Unclassified bacteria	Unclassified bacteria	Unclassified bacteria	1	95	95
superkingdom	Num. phylum	Num. class	Num. order	Num. family	Num. genus	Num. species	Num. Blast	Max% Blast	Min%	
Bacteria	89	2	1	1	1	1	1	100	100	100
			Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	Escherichia coli	1	100	100
							Raistonia oxalatica	1	89	89
							Clostridium ramosum	9	89	89
							Clostridium sporforme	1	84	91
							Streptococcus salivarius	1	100	100
							Clostridium papyrosolvens	7	88	88
							Clostridium viride	2	94	91
							Clostridium hathewayi	2	96	96
							Clostridium boltaeae	1	95	95
							Clostridium boletaeae	4	99	99
							Dorea formicigenans	2	99	99
							Dorea longicatena	2	99	99
							Faecalibacterium prausnitzii	4	98	91
							Ruminococcus productus	3	98	98
							Ruminococcus granus	27	100	99
							Ruminococcus obeum	3	98	98
							Ruminococcus hydrogencrophiicus	14	100	99
							Ruminococcus luti	4	97	92
							Ruminococcus shinkii	5	97	97
							Ruminococcus luti	5	98	97
							Anaerostipes caecae	1	89	86
							Anaerostipes caecae	1	86	86
							Eubacterium desmoliens	3	97	93
							Eubacterium rectale	1	99	99
							Bacteroides vulgatus	28	98	96
							Bacteroides ovatus	4	98	93
							Bacteroides stercoris	16	99	90
							Bacteroides caecae	1	96	96
							Collinsella aerofaciens	3	99	97
89	Actinobacteria	89	3	3	3	3	3	89	89	97
			Actinobacteria (class)	Coriobacteriales	Coriobacteriaceae	Collinsella	Collinsella aerofaciens	3	89	97













Intestinal bacterial flora count and destruction rate after oral administration of preB®

	Weight	Color	Hardness	Quantity of food residue	Flora count before test	Flora count after test	Destruction rate (%)
1	0.100	Dark brown	Hard	Small	$7.4 \times 10^{10}$	$4.1 \times 10^9$	83.2
2	0.100	Brown	Average	Small	$6.9 \times 10^{10}$	$4.9 \times 10^8$	78.9
3	0.100	Greenish brown	Soft	Small	$9.6 \times 10^9$	$1.7 \times 10^8$	94.8
4	0.100	Brown	Average	Average	$5.8 \times 10^{10}$	$1.7 \times 10^9$	91.2
5	0.100	Brown	Soft	Average	$6.9 \times 10^{10}$	$2.6 \times 10^9$	88.8
6	0.100	Ocher	Soft	Small	$7.4 \times 10^{10}$	$3.0 \times 10^9$	87.7
7	0.100	Brown	Average	Average	$4.8 \times 10^{10}$	$1.9 \times 10^9$	88.5
8	0.100	Ocher	Soft (partly sludged)	Small	$5.5 \times 10^{10}$	$2.2 \times 10^9$	87.8
9	0.100	Brown	Average	Small	$8.2 \times 10^{10}$	$9.5 \times 10^8$	96.5
10	0.100	Brown	Soft	Average	$6.5 \times 10^{10}$	$2.4 \times 10^9$	88.8
11	0.101	Brown	Hard	Average	$7.0 \times 10^{10}$	$8.4 \times 10^8$	96.4
12	0.099	Brown	Soft	Large	$4.3 \times 10^{10}$	$1.8 \times 10^8$	98.7
13	0.100	Ocher	Soft	Average	$4.3 \times 10^{10}$	$2.7 \times 10^8$	98.2
14	0.100	Ocher	Soft	Average	$1.1 \times 10^{10}$	$1.2 \times 10^8$	96.8
15	0.097	Dark brown	Hard	Small	$1.1 \times 10^{11}$	$7.5 \times 10^7$	99.8
16	0.097	Brown	Hard	Small	$7.4 \times 10^{10}$	$1.3 \times 10^9$	94.9
17	0.107	Brown	Soft	Average	$3.8 \times 10^{10}$	$3.3 \times 10^8$	97.4
18	0.101	Ocher	Average	Small	$5.2 \times 10^{10}$	$5.7 \times 10^8$	96.7
19	0.099	Dark brown	Hard	Average	$7.2 \times 10^{10}$	$3.4 \times 10^8$	98.6
20	0.101	Brown	Soft	Average	$5.4 \times 10^{10}$	$5.6 \times 10^8$	96.9
21	0.035	Greenish brown	Soft	Small	$3.1 \times 10^{10}$	$9.0 \times 10^7$	99.1
22	0.103	Brown	Average	Average	$4.5 \times 10^{10}$	$1.3 \times 10^9$	91.5
23	0.102	Brown	Hard	Average	$4.8 \times 10^{10}$	$7.3 \times 10^8$	95.4
24	0.098	Brown	Soft	Average	$4.0 \times 10^{10}$	$1.5 \times 10^9$	88.8
25	0.101	Brown	Soft	Average	$3.5 \times 10^{10}$	$1.2 \times 10^9$	89.7
26	0.091	Dark brown	Hard	Large	$7.1 \times 10^{10}$	$2.3 \times 10^9$	90.4
27	0.098	Greenish brown	Soft	Large	$3.2 \times 10^{10}$	$5.7 \times 10^7$	99.5
28	0.099	Greenish brown	Soft	Small	$3.4 \times 10^{10}$	$1.4 \times 10^8$	98.8
29	0.098	Brown	Soft	Small	$7.6 \times 10^{10}$	$1.5 \times 10^9$	94.1
30	0.101	Brown	Soft	Average	$6.7 \times 10^{10}$	$2.5 \times 10^9$	89.0







no. 7		no. 8	
superkingdom	Num	superkingdom	Num
Bacteria	94	Bacteria	82
phylum		phylum	
Firmicutes	67	Firmicutes	82
class		class	
"Erysipelotrichi"	2	"Erysipelotrichi"	82
order		order	
"Erysipelotrichales"	2	"Erysipelotrichales"	82
family		family	
Erysipelotrichaceae	2	Erysipelotrichaceae	82
genus		genus	
Erysipelotrichaceae Incertae Sedis	2	Erysipelotrichaceae Incertae Sedis	82
species		species	
Clostridium sporforme	2	Clostridium sporforme	82
Eubacterium dolichum	1	Eubacterium dolichum	82
Bacillus subtilis	1	Bacillus subtilis	82
Megaspheera microformis	2	Megaspheera microformis	82
Dialister invius	2	Dialister invius	82
Mitsuokella	1	Mitsuokella	82
Clostridium celerecens	2	Clostridium celerecens	82
Clostridium xylanolyticum	1	Clostridium xylanolyticum	82
Clostridium viride	1	Clostridium viride	82
Clostridium acetivulgens	2	Clostridium acetivulgens	82
Clostridium methylpentosum	1	Clostridium methylpentosum	82
Clostridium saccharolyticum	1	Clostridium saccharolyticum	82
Clostridium botkiae	5	Clostridium botkiae	82
Sporobacter	1	Sporobacter	82
Sporobacter tennitidis	1	Sporobacter tennitidis	82
Dorea longicatena	1	Dorea longicatena	82
Faecalibacterium	14	Faecalibacterium	82
Faecalibacterium prausnitzii	14	Faecalibacterium prausnitzii	82
Ruminococcus productus	1	Ruminococcus productus	82
Ruminococcus obeum	8	Ruminococcus obeum	82
Ruminococcus hydrogenophilicus	1	Ruminococcus hydrogenophilicus	82
Ruminococcus luti	1	Ruminococcus luti	82
Lachnospira pectinoshiza	1	Lachnospira pectinoshiza	82
Anaerostipes caecae	3	Anaerostipes caecae	82
Eubacterium desmolans	2	Eubacterium desmolans	82
Eubacterium eligens	2	Eubacterium eligens	82
Eubacterium hallii	4	Eubacterium hallii	82
Bacteroides fragilis	4	Bacteroides fragilis	82
Bacteroides vulgatus	1	Bacteroides vulgatus	82
Bacteroides ovatus	3	Bacteroides ovatus	82
Bacteroides stercoris	1	Bacteroides stercoris	82
Bacteroides caecae	3	Bacteroides caecae	82
Prevotellaceae	1	Prevotellaceae	82
Coriobacteriales	1	Coriobacteriales	82
Prevotella	17	Prevotella	82
Collinsella	1	Collinsella	82
phylum		phylum	
Actinobacteria	94	Actinobacteria	82
class		class	
Actinobacteria (class)	94	Actinobacteria (class)	82
order		order	
Coriobacteriales	1	Coriobacteriales	82
family		family	
Coriobacteriaceae	1	Coriobacteriaceae	82
genus		genus	
Coriobacteriaceae Incertae Sedis	2	Coriobacteriaceae Incertae Sedis	82
species		species	
Clostridium sporforme	2	Clostridium sporforme	82
Eubacterium bifforme	2	Eubacterium bifforme	82
Clostridium sporforme	1	Clostridium sporforme	82
Streptococcus sinensis	1	Streptococcus sinensis	82
Lactobacillus salivarius	1	Lactobacillus salivarius	82
Papillibacter cinnamivorans	1	Papillibacter cinnamivorans	82
Clostridium fluseburvense	9	Clostridium fluseburvense	82
Clostridium irregulare	4	Clostridium irregulare	82
Clostridium dispersum	1	Clostridium dispersum	82
Clostridium saccharolyticum	3	Clostridium saccharolyticum	82
Sporobacter	1	Sporobacter	82
Sporobacter tennitidis	1	Sporobacter tennitidis	82
Faecalibacterium	11	Faecalibacterium	82
Faecalibacterium prausnitzii	11	Faecalibacterium prausnitzii	82
Roseburia intestinalis	1	Roseburia intestinalis	82
Ruminococcus albus	1	Ruminococcus albus	82
Ruminococcus flavafaciens	2	Ruminococcus flavafaciens	82
Ruminococcus bromii	1	Ruminococcus bromii	82
Ruminococcus obeum	2	Ruminococcus obeum	82
Lachnospira pectinoshiza	2	Lachnospira pectinoshiza	82
Anaerostipes caecae	3	Anaerostipes caecae	82
Eubacterium eligens	12	Eubacterium eligens	82
Eubacterium hallii	1	Eubacterium hallii	82
Eubacterium rectale	10	Eubacterium rectale	82
Bacteroides vulgatus	27	Bacteroides vulgatus	82
Bacteroides distans	2	Bacteroides distans	82
Bacteroides ovatus	1	Bacteroides ovatus	82
Bacteroides stercoris	22	Bacteroides stercoris	82
Bacteroides caecae	1	Bacteroides caecae	82
Collinsella aerofaciens	2	Collinsella aerofaciens	82
Uncultured bacterium clone, F2	1	Uncultured bacterium clone, F2	82
Num	88	Num	88
Max%	93	Max%	93
Min%	96	Min%	96







no.	superkingdom	Num	phylum	Num	class	Num	order	Num	family	Num	genus	Num	species	Num	Max%	Min%
no. 15	Supra	89	Firmicutes	51	Erysipelotrichi	7	Erysipelotrichales	7	Erysipelotrichaceae	7	Erysipelotrichaceae Incertae Sedis	6	Streptococcus pleiomorphus	1	100	100
					Bacilli	2	Bacillales	1	Turichobacteraceae	1	Holdemania	1	Clostridium sporforme	5	90	90
					Clostridia	42	Clostridiales	42	Lactobacillaceae	1	Turichobacter	1	Holdemania filiformis	1	95	95
									Clostridiaceae	1	Lactobacillus	1	Lactobacillus sanguinis	1	99	99
										14	Clostridium	13	Clostridium crispatus	1	99	99
												1	Clostridium cellulyticum	3	89	88
												1	Clostridium celerecrescens	1	97	97
												1	Clostridium colinum	1	95	95
												1	Clostridium methyloferosum	1	88	88
												3	Clostridium orbischiense	3	99	92
												2	Clostridium hyemomae	2	96	95
												1	Clostridium hathewayi	1	95	95
												1	Clostridium boltaeae	1	95	95
												1	Faecalibacterium prausnitzii	1	92	92
												23	Ruminococcus productus	2	98	97
												9	Ruminococcus torques	9	96	94
												9	Ruminococcus lactaris	9	98	98
												1	Ruminococcus bromii	1	96	96
												1	Ruminococcus lactarius	1	96	96
												5	Ruminococcus hydrogenotrophicus	5	96	96
												1	Ruminococcus schinkii	1	97	97
												5	Eubacterium hallii	5	96	96
												1	Bacteroides thetaiotaomicron	1	98	98
												5	Bacteroides vulgatus	5	98	98
												3	Bacteroides distasonis	3	99	99
												2	Bacteroides ovatus	2	99	98
												2	Bacteroides merdae	2	99	99
												2	Bacteroides stercoris	2	94	90
												3	Megamonas hypermegale	3	95	95
												8	Alistipes putredinis	8	94	93
												2	Collinsella aerofaciens	2	99	99
												1	Collinsella intestinalis	1	94	94
no. 16	Supra	89	Firmicutes	63	Erysipelotrichi	2	Erysipelotrichales	2	Erysipelotrichaceae	2	Erysipelotrichaceae Incertae Sedis	2	Clostridium spiriforme	2	91	91
					Bacilli	2	Lactobacillales	2	Streptococcaceae	2	Streptococcus	2	Streptococcus salivarius	1	100	100
					Clostridia	61	Clostridiales	61	Clostridiaceae	31	Clostridium	20	Clostridium leptum	5	97	97
												4	Clostridium kluseburrense	4	97	97
												3	Clostridium sporosphaeroides	3	93	92
												1	Clostridium nexile	1	97	97
												1	Clostridium indolis	1	88	88
												4	Clostridium orbischiense	4	94	92
												2	Clostridium boltaeae	2	99	97
												1	Dorea longicatena	1	99	99
												10	Faecalibacterium prausnitzii	10	98	92
												4	Roseburia intestinalis	4	100	96
												1	Ruminococcus flavifaciens	1	89	89
												3	Ruminococcus bromii	3	98	98
												11	Ruminococcus obeum	11	97	96
												1	Ruminococcus lactaris	1	95	95
												4	Ruminococcus hydrognotrophicus	4	97	96
												1	Lachnospira pectinivorans	1	98	98
												1	Anaerofilum pentosovorans	1	90	90
												4	Eubacterium desmoulans	4	98	98
												2	Eubacterium elligenis	2	92	92
												1	Eubacterium hallii	1	96	96
												2	Bacteroides fragilis	2	100	100
												6	Bacteroides vulgatus	6	98	98
												5	Bacteroides caecae	5	97	96
												5	Alistipes putredinis	5	94	89
												2	Olsenella lili	2	95	95
85	Actinobacteria	2	Actinobacteria (Class)	2	Coriobacteriales	2	Coriobacteriales	2	Coriobacteriaceae	2	Olsenella	2	Olsenella lili	2	95	95



no.	superkingdom	Num	phylum	Num	class	Num	order	Num	family	Num	genus	Num	species	Num	Max%	Min%
no. 19	superkingdom	88	phylum	62	"Erysiplotrichi"	5	Erysiplotrichiaceae	5	Erysiplotrichiaceae	5	Erysiplotrichiaceae	5	5	94	94	
	Bacteria	88	Firmicutes	62	Bacilli	6	Lactobacillales	6	Streptococcaceae	6	Streptococcus	6	6	100	99	
					Clostridia	51	Clostridiales	51	Actinomonococcaceae	51	Phaeoclostracterium	1	1	100	100	
									Clostridiaceae	29	Clostridium	29	29	88	88	
										1	Clostridium cellulosilyticum	1	1	89	88	
										4	Clostridium leptum	4	4	97	97	
										1	Clostridium schindleri	1	1	100	100	
										5	Clostridium viride	5	5	91	91	
										1	Clostridium viride	1	1	83	83	
										1	Clostridium hylemonae	1	1	86	86	
										1	Coprobacillus cateriniformis	1	1	88	88	
										12	Faecalibacterium prausnitzii	12	12	92	92	
										14	Ruminococcus albus	14	14	95	95	
										1	Ruminococcus gnavus	1	1	100	100	
										1	Ruminococcus longus	1	1	99	99	
										4	Ruminococcus bromii	4	4	98	98	
										5	Ruminococcus obeum	5	5	97	96	
										1	Ruminococcus hydrogenotrophicus	1	1	97	97	
										1	Ruminococcus schinkii	1	1	97	97	
										2	Anaeroflum parvivorans	2	2	88	88	
										2	Anaeroflum caecae	2	2	89	89	
										4	Eubacterium aerophilum	4	4	88	88	
										4	Eubacterium	4	4	88	88	
										2	Eubacterium hallii	2	2	88	88	
										1	Eubacterium ventriosum	1	1	94	94	
										17	Bacteroides vulgatus	17	17	89	89	
										1	Bacteroides distans	1	1	89	89	
										3	Bacteroides merdae	3	3	99	99	
										3	Bacteroides stercoris	3	3	99	99	
										3	Bacteroides caecae	3	3	99	97	
										1	Bacteroides acidifaciens	1	1	98	98	
										1	Megamonas hypermegalae	1	1	95	95	
										7	Collinsella serotrans	7	7	99	99	
										1	Actinomyces bowditchii	1	1	91	91	
										88		88	88			
no. 20	superkingdom	88	phylum	88	Actinobacteria (class)	8	Coriobacteriales	8	Coriobacteriaceae	7	Megamonas	7	7	99	99	
	Bacteria	88	Firmicutes	88	Clostridia	69	Clostridiales	69	Actinomycetaceae	69	Actinomycetes	69	69	99	99	
										2	Rhodospirillum rubrum	2	2	85	85	
										3	Actinomyces fermentans	3	3	86	86	
										2	Megamonas	2	2	88	88	
										1	Phaeoclostracterium faecium	1	1	100	100	
										12	Clostridium cellulosilyticum	12	12	90	90	
										1	Clostridium cellobiosorum	1	1	88	85	
										1	Clostridium xylanolyticum	1	1	94	94	
										1	Clostridium viride	1	1	97	97	
										1	Clostridium obscurans	1	1	95	95	
										1	Clostridium saccharolyticum	1	1	95	95	
										3	Clostridium hathewayi	3	3	96	95	
										2	Dorea longicatena	2	2	99	99	
										1	Faecalibacterium prausnitzii	1	1	93	93	
										3	Roseburia intestinalis	3	3	100	99	
										16	Ruminococcus gnavus	16	16	100	100	
										1	Ruminococcus torques	1	1	99	98	
										3	Ruminococcus obeum	3	3	99	98	
										1	Ruminococcus hydrogenotrophicus	1	1	89	88	
										3	Ruminococcus lhti	3	3	86	86	
										1	Lachnospira pactinoschizans	1	1	86	86	
										18	Eubacterium eligens	18	18	92	92	
										1	Eubacterium hallii	1	1	82	82	
										15	Eubacterium rectale	15	15	99	97	
										1	Clostridium rectum	1	1	98	98	
										7	Bacteroides vulgatus	7	7	98	98	
										2	Bacteroides distans	2	2	98	86	
										1	Bacteroides caecae	1	1	99	99	
										2	Bacteroides acidifaciens	2	2	96	95	
										2	Megamonas hypermegalae	2	2	97	97	
										5	Alistipes putredinis	5	5	97	97	
										1	Tannerella forsythensis	1	1	87	87	
										4	Collinsella serotrans	4	4	99	98	
										2	Eubacterium angustum	2	2	99	98	
										3	Unclassified bacterium aduifae04	3	3	99	95	
										3	Unclassified bacterium aduifae12,25	3	3	97	92	
										2	Unclassified bacterium aduifae12,25	2	2	97	92	

no. 21	superkingdom	Num	phylum	Num	class	Num	order	Num	family	Num	genus	Num	species	Num	Max%	Min%
86	Bacteria	94	Firmicutes	61	"Erysipelotrichi"	18	Erysipelotrichales	18	Erysipelotrichaceae	18	Erysipelotrichaceae Incertae Sedis	18	Clostridium spiroforme	1	94	93
					Clostridia	43	Clostridiales	43	Clostridiaceae	18	Clostridium	6	Clostridium disporicum	1	95	100
													Clostridium hathewayi	4	95	95
													Clostridium holtsae	5	99	97
													Dorea longicatena	7	98	97
													Faccalibacterium praeutilizii	6	98	96
													Ruminococcus productus	4	100	100
													Ruminococcus gnavus	3	97	95
													Ruminococcus obeum	1	97	97
													Ruminococcus hydrogenotrophicus	5	98	95
													Ruminococcus schinkii	2	96	96
													Anaerostipes caccae	2	98	98
													Eubacterium hallii	2	99	99
													Eubacterium rectale	2	99	99
													Bacteroides vulgatus	23	98	97
													Bacteroides distasonis	5	97	97
													Bacteroides ovatus	2	99	99
													Bacteroides stercoris	1	98	98
													Bacteroides caccae	1	99	99
													Bifidobacterium angulatum	1	99	99
86		86		86		86		86		86		86		86		86
no. 22	superkingdom	Num	phylum	Num	class	Num	order	Num	family	Num	genus	Num	species	Num	Max%	Min%
94	Bacteria	95	Firmicutes	60	"Erysipelotrichi"	10	Erysipelotrichales	10	Erysipelotrichaceae	10	Erysipelotrichaceae Incertae Sedis	10	Clostridium apiforme	1	99	99
					Bacilli	8	Bacillales	8	Bacillaceae	1	Bacillus	1	Bacillus subtilis	6	99	99
													Streptococcus litetensis	3	100	99
													Streptococcus salivarius	3	100	99
													Acidimicrococcus fermentans	1	97	97
													Phascolarctobacterium rasedum	1	94	94
													Clostridium celerescens	4	94	94
													Clostridium xyanoformans	1	95	96
													Clostridium hathewayi	2	95	96
													Phascolarctobacterium praeutilizii	7	95	95
													Rosaburra intrastrials	1	98	98
													Ruminococcus productus	2	98	96
													Ruminococcus gnavus	2	100	100
													Ruminococcus torques	1	96	96
													Ruminococcus obeum	6	99	96
													Ruminococcus hydrogenotrophicus	2	97	96
													Ruminococcus luti	4	98	97
													Ruminococcus schinkii	1	97	97
													Lachnospira pectinoschiza	1	96	96
													Anaerostipes caccae	8	96	96
													Syntrophococcus sucromutans	1	92	92
													Eubacterium rectale	1	99	99
													Bacteroides vulgatus	1	98	98
													Bacteroides distasonis	1	97	97
													Bacteroides stercoris	4	90	90
													Bacteroides caccae	1	95	95
													Bacteroides melaninogenicus	1	90	90
													Prevotella outlerum	8	90	90
													Prevotella bryantii	18	93	92
													Prevotella bryantii	2	91	91
95		95		95		95		95		95		95		95		95







no.	Superkingdom	Num	phylum	Num	class	Num	order	Num	family	Num	genus	Num	species	Num	Max%	Min%
29	Bacteria	91	Firmicutes	52	"Erysipelotrichi"	2	"Erysipelotrichales"	2	Erysipelotrichaceae	2	Erysipelotrichaceae	2	Eubacterium bifforme	2	98	98
					Clostridia	50	Clostridiales	50	Acidaminococcaceae	2	Acidaminococcus	1	Acidaminococcus fermiretus	1	90	90
									Clostridiaceae	18	Phascolarctobacterium	1	Phascolarctobacterium faecium	1	94	94
											1	Clostridium calluoyticum	1	86	86	
											1	Clostridium irregulare	1	98	98	
											1	Clostridium xyanoilyticum	1	94	94	
											1	Clostridium hadweyvi	1	95	95	
											13	Clostridium lactatifermentans	13	99	92	
											1	Faecalibacterium prausnitzii	1	90	90	
											14	Ruminococcus flavofaciens	4	93	93	
											8	Ruminococcus obeum	8	99	95	
											3	Ruminococcus hydrogenotrophicus	3	97	97	
											2	Catenibacterium mitsuokai	2	96	96	
											2	Anaerostipes caccae	2	96	96	
											10	Eubacterium desmolans	10	96	96	
											9	Eubacterium rectale	9	99	98	
											9	Bacteroides vulgatus	9	99	98	
											3	Bacteroides distasonis	3	99	94	
											1	Bacteroides merdae	1	94	94	
											2	Bacteroides caccae	2	96	95	
											1	Aliistipes putredinis	1	97	97	
											3	Prevotella melanogenica	3	90	90	
											24	Prevotella outorum	24	93	92	
											1	Prevotella bryantii	1	92	92	
											1	Collinsella aerofaciens	1	99	99	
30	Bacteria	93	Firmicutes	64	"Erysipelotrichi"	1	"Erysipelotrichales"	1	Erysipelotrichaceae	1	Erysipelotrichaceae	1	Ooerstridium ramosum	1	99	99
					Bacilli	2	Lactobacillales	2	Sporobolaceae	2	Sporobolus	2	Sporobolus salivarius	2	99	99
					Clostridia	61	Clostridiales	61	Acidaminococcaceae	1	Vellionella	1	Vellionella parvula	1	99	99
									Clostridiaceae	25	Clostridium	6	Clostridium leparum	1	90	90
											1	Clostridium irregulare	1	98	98	
											1	Clostridium sphenoides	1	95	95	
											1	Clostridium orbiscindens	1	94	94	
											1	Clostridium saccharolyticum	1	96	96	
											19	Clostridium hyemoneae	19	98	93	
											3	Faecalibacterium prausnitzii	3	100	99	
											3	Roseburia intestinalis	3	98	98	
											13	Ruminococcus bromii	2	98	98	
											2	Ruminococcus callidus	2	90	86	
											3	Ruminococcus obeum	3	97	96	
											3	Ruminococcus hydrogenotrophicus	3	99	94	
											3	Anaerostipes caccae	3	98	98	
											16	Eubacterium eligens	3	98	98	
											3	Eubacterium hallii	4	99	97	
											4	Eubacterium siraeum	4	97	97	
											1	Eubacterium ruminantium	1	96	96	
											29	Bacteroides vulgatus	8	99	96	
											1	Bacteroides distasonis	1	98	98	
											4	Bacteroides ovatus	4	93	93	
											5	Bacteroides stercoris	5	99	94	
											8	Bacteroides caccae	8	97	95	
											3	Bacteroides acidifaciens	3	98	98	