Dr Henry Butt MSc PhD



Specialist Microbiological Laboratory
ABN: 87 682 058 987

5 Little Hyde Street Yarraville, Victoria, 3013 Ph: +61 3 9687 3355 Fax: +61 3 9687 3377

admin@bioscreenmedical.com

Report of Faecal Microbiology

Patient Name: Vihaan DODDI

Address: 9 Lambourne Ave

Truganina VIC 3029

Date of Birth: 23/09/2014

Name of Requesting Practitioner: Joe NASTASI (BRUNSWICK)

Laboratory Number:153709Date of Sample Collection:5/07/2021Date of Sample Processing:06/07/2021Date of Report Issued:19/07/2021

Dear Dr Joe NASTASI (BRUNSWICK),

Thank you for referring your patient to Bioscreen.

The following is a summary of our faecal microbiota testing from your patient's sample.



Detailed Report, Faecal Microbiology

Bacterial Count (Total)
Facultative Anaerobe
[Aerobe] Counts
Anaerobe Count

Count cfu/g	Counts Reference Range cfu/g	Comment	Distribution % Total Count	Distribution Reference Range
2.5 x 10 ¹⁰	1.0 x 10 ⁹ - 1.0 x 10 ¹²	Within Ref Range		
5.8 x 10 ⁷	1.0 x 10 ⁷ - 1.0 x 10 ⁸	Within Ref Range		
2.5 x 10 ¹⁰	1.0 x 10 ⁸ - 1.0 x 10 ¹²	Within Ref Range		

Aerobe: Anaerobe Ratio:

2.3 (Reference Range is 0.5 - 4.0)

Facultative Anaerobe [Aerobe] Counts

Aerobe Count (total) Escherichia coli coliform (Total)

Escherichia coli

Enterococcus (Total)

Enterococcus faecalis

Streptococcus (Total)

Streptococcus agalactiae

Streptococcus salivarius

Other aerobes

Rothia dentocariosa

Count cfu/g	Counts Reference Range cfu/g	Comment	Distribution % Total Count	Distribution Reference Range
5.8 x 10 ⁷	1.0 x 10 ⁷ - 1.0 x 10 ⁸	Within Ref Range		
2.7 x 10 ⁶	7.0 x 10 ⁶ - 9.0 x 10 ⁷	Low		
2.7 x 10 ⁶			4.7%	70-90%
2.7 x 10 ⁵	<5.0 x 10 ⁵	Within Ref Range	0.5%	<5%
2.7 x 10 ⁵			0.5%	
5.5 x 10 ⁷	<3.0 x 10 ⁵	High	94.8%	<5%
9.1 x 10 ⁴			0.2%	
5.5 x 10 ⁷			94.6%	
	<1.0 x 10 ⁵			<5%
9.1 x 10 ³	<1.0 x 10 ⁵	Within Ref Range	< 0.01%	

Faecal Fungi (Total)

Candida parapsilosis

Count cfu/g	Counts Reference Range cfu/g	Comment	Distribution % Total Count	Distribution Reference Range
1.8 x 10 ³	<1.0 x 10 ⁴	Within Ref Range		
1.8 x 10 ³				

Specialist Microbiological Laboratory

ABN: 87 682 058 987

Anaerobe Counts

Anaerobe Count (total)

Bacteroides and related genera (Total)

Bacteroides thetaiotaomicron

Bacteroides uniformis

Bacteroides ovatus

Eubacterium and related genera (Total)

Collinsella aerofaciens

Lactobacillus and related genera (Total)

Bifidobacterium and related genera (Total)

Bifidobacterium longum

Other anaerobes

Catenibacterium mitsuokai Acidaminococcus intestini

Count cfu/g	Counts Reference Range cfu/g	Comment	Distribution % Total Count	Distribution Reference Range
2.5 x 10 ¹⁰	1.0 x 10 ⁸ - 1.0 x 10 ¹²	Within Ref Range		
		Within Ref Range		
1.2 x 10 ¹⁰	5.0 x 10 ⁸ - 9.5 x 10 ¹¹	Limited Species Detected	47.8%	85-95%
9.1 x 10 ⁹			37%	
2.5 x 10 ⁹			10.4%	
9.1 x 10 ⁷			0.4%	
9.1 x 10 ⁹	1.0 x 10 ⁸ - 1.0 x 10 ⁹	High	37%	<15%
9.1 x 10 ⁹			37%	
<5.0 x 10 ⁵	5.0 x 10 ⁵ - 1.0 x 10 ⁷	Low	< 0.01%	0.5-2%
2.7 x 10 ⁹	5.0 x 10 ⁵ - 5.0 x 10 ⁸	High	11.1%	5-11%
2.7 x 10 ⁹			11.1%	
	<5.0 x 10 ⁸			1-10%
9.1 x 10 ⁸	<5.0 x 10 ⁸	High	3.7%	
9.1 x 10 ⁷	<5.0 x 10 ⁸	Within Ref Range	0.4%	

Examples of Scientific Notation of Powers of Ten

 $10^2 = 10 \times 10 = 100$

 $10^3 = 10 \times 10 \times 10 = 1,000$

 $3.5 \times 10^2 = 3.5 \times 10 \times 10 = 3500$



Summary Report, Faecal Microbiology

Total Aerobe Count: 5.8 x 10⁷ cfu/g (colony forming units/g)

Total Anaerobe Count: 2.5 x 1010 cfu/g

Aerobe: Anaerobe Ratio is: 2.3. The Reference Range is 0.5 to 4.

Comment: This ratio is within the reference range.

Faecal Aerobes

Streptococcus sp.: Overgrowth

E.Coli: Undergrowth

Comments

E. coli

- The reason for the low E.coli percentage distribution/ total count in the sample is unclear. However, recent exposure to antipyretics and/or analgesia (eg. paracetamol) may cause a marked change in the faecal ecology resulting in a significant alteration of the E.coli viable count (Bioscreen data, 2001). Recent supplementation with fructo-oligosaccharide (FOS) may also have suppressed growth of this organism.
- Undetectable levels of E.coli. Oral application of E.coli probiotics may be beneficial. Changing and normalizing the colonic aerobic microbial flora with the E. coli probiotic has shown to be safe and beneficial in patients with Ulcerative Colitis^{1,2} and Crohn's Disease³. The probiotic, once ingested and if adhere to the mucosal wall, will colonize the colon within a few days, and will remain colonized after oral administration ceased. Oral application of the E.coli probiotic has shown to stimulate and enhance immune responses and induces nonspecific natural immunity^{4,5}.
- E.coli is an important intestinal micro-organism responsible for the synthesis of essential amino acids (eg. trytophan, phenylalanine, tyrosine)^{6,7,8} vitamins (folic acid, vit K2)^{9,10}, and coenzymes (CoQ10)¹¹ important for cellular metabolism and reproduction. Determination into the levels of these essential amino acids in patients with persistent and chronic low levels of E.coli may be beneficial. Acute depletion of tyrosine and phenylalanine has shown to have selective effect on decision-making in depressive patients¹². Tyrosine depletion has also shown to have recognition and working memory impairment¹³.
- Consider supplementing oral sugars (eg galactose, fucose) to increase the densities of current intestinal coliforms (eg E.coli)^{14,15} as opposed to adding a different strain with probiotics. Health professionals can contact Bioscreen for further information.
- Consider checking the folate, vitamin K2, CoQ10 levels and supplement if indicated.
- Consider checking the levels of the following essential amino acids: tryptophan, tyrosine, phenylalanine, and supplement if indicated.

Streptococcus/Enterococcus

- Streptococcus spp. are Gram positive, facultative anaerobic organisms and are classified as homofermenative, producing only lactic acid from glucose catabolism and generally regarded as potent D- and L-lactic acid producers (Bioscreen data).
- Increased distribution of lactic acid bacteria (Streptococcus, Enterococcus sp.) may lower the colonic pH¹⁶ and has been reported to :(1) modify faecal microbial metabolism particularly the Bacteroides and Bifidobacterium spp, resulting in a decreased production of volatile fatty acids¹⁷, and (2) alter intestinal epithelial barrier function increasing passive intestinal permeability to small and large molecules. However, this consideration requires further study.
- High colonization of faecal lactic acid bacteria (Streptococcus, Enterococcus sp.) significantly and positively correlate with cognitive dysfunctions (nervousness, memory loss, forgetfulness, confusion, mind going blank)^{18,19,20,21}, and sleep patterns (Bioscreen data).



- Increased proportion of lactic acid may result in a change in the distribution of the anaerobic microbial flora. This change of the fecal flora may affect the production of primary bile acids and influencing the bile acid composition in both the bile and the intestine²². The possibility of fat malabsorption may occur. However, this consideration requires further study.
- If indicated, erythromycin may assist in the suppression of the faecal Streptococcus spp.
 Ampicillin/amoxycillin may be a suitable alternative if patient is reported to have adverse reactions to the macrolids.

Faecal Anaerobes

Eubacterium sp.: Overgrowth Bifidobacterium sp.: Overgrowth Catenibacterium sp.: Overgrowth

Bacteroides sp.: Limited Bacteroides spp. detected

Lactobacillus sp.: Undergrowth

Comments

Bacteroides sp.

- Limited and low distribution of Bacteroides spp may affect the availability of volatile fatty acids for cellular metabolism.
- Consider the supplement of menaquinones (vitamin K2), and protoporphyrin IX (hemin) to improve the growth of anaerobes. High concentration of vitamin K2 (up to 300µg/kg) is found in natural yogurt, cheese, and butter²³.
- Consider the supplement of biotin, bicarbonate, sodium and/or potassium to assist the production of volatile fatty acids by anaerobes.
- A general diet consisting of meat/bone broth, fish protein or soybean protein may assist the growth of these organisms
- This change of the fecal flora may affect the production of primary bile acids and influencing the bile acid composition in both the bile and the intestine²².

Bifidobacterium/Lactobacillus sp.

- Members of the genus Bifidobacterium are Gram positive branching bacilli and lactic acid producing bacteria. A few members can grow in a microaerophilic environment; but most are obligate anaerobes.
- High levels of Bifidobacterium spp. in the anaerobic microbial flora. Increased level of Bifidobacterium may stimulate amine production²⁴. Similarly, increased levels of this organism may also lower the colonic pH¹⁶, modifying faecal microbial metabolism particularly the Bacteroides spp, resulting in a decreased production of volatile fatty acids¹⁷, and altering intestinal epithelial barrier function increasing passive intestinal permeability to small and large molecules.
- Cease all oral supplementation of lactic acid probiotics if indicated. If required, consider an antimicrobial agent (eg. ampicillin) to assist in the suppression of the organisms Bifidobacterium spp.
- Members of the genus Lactobacillus are Gram positive bacilli and lactic acid producing bacteria. A few members can grow in a microaerophilic environment; but most are obligate anaerobes.
- Low levels of Lactobacillus spp. detected. Oral Supplementation of Lactobacillus probiotics may be beneficial. Consider the intake of vitamin B6 and biotin to promote lactobacilli growth^{25,26}.

Eubacterium sp.

- Eubacterium sp is generally regarded as one of the most frequently recovered organisms in the gastrointestinal tract, second only to Bacteroides spp.
- The increased distribution of this organism in the gastrointestinal tract is unclear, however, the cell wall of the organism has shown to be proinflammatory and arthritogenic²⁷.



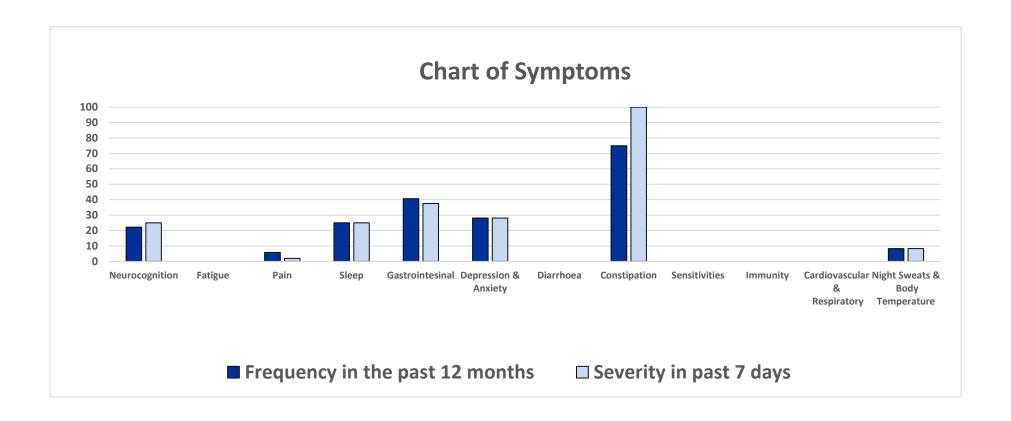
Catenibacterium sp.

• A strict Gram positive anaerobe and a member of the human gut commensal organism. Fermentation products of glucose are acetic, lactic, butyric and isobutyric acids²⁸.

Faecal Fungi

• Faecal fungi were recovered and the % abundance was within the normal range.

ABN: 87 682 058 987





If you require further assistance please contact Bioscreen and arrange a consultation.

Report authorised 20th July, 2021 by Dr Henry Butt.

Bioscreen Pty Ltd.

References

- 1. Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. Lancet. 1999 Aug 21;354(9179):635-9.
- 2. Kruis W, Schütz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther. 1997 Oct;11(5):853-8.
- 3. Malchow HA. Crohn's disease and Escherichia coli. A new approach in therapy to maintain remission of colonic Crohn's disease? J Clin Gastroenterol. 1997 Dec;25(4):653-8.
- 4. Cukrowska B, Lodlnová-Zádnlková R, Enders C, Sonnenborn U, Schulze J, Tlaskalová-Hogenová H. Specific proliferative and antibody responses of premature infants to intestinal colonization with nonpathogenic probiotic E. coli strain Nissle 1917. Scand J Immunol. 2002 Feb;55(2):204-9.
- 5. Hockertz S. Immunomodulating effect of killed, apathogenic Escherichia coli, strain Nissle 1917, on the macrophage system. Arzneimittelforschung. 1991 Oct;41(10):1108-12.
- 6. Dosselaere F, Vanderleyden J. A metabolic node in action: chorismate-utilizing enzymes in microorganisms. Crit Rev Microbiol 2001;27.
- 7. Gerigk M, Bujnicki R, Ganpo-Nkwenkwa E, Bongaerts J, Sprenger G, Takors R. Process control for enhanced L-phenylalanine production using different recombinant Escherichia coli strains. Biotechnol Bioeng 2002;80.
- 8. Polen T, Kramer M, Bongaerts J, Wubbolts M, Wendisch VF. The global gene expression response of Escherichia coli to L-phenylalanine. J Biotechnol 2005;115.
- 9. Roux B, Walsh CT. p-Aminobenzoate synthesis in Escherichia coli: mutational analysis of three conserved amino acid residues of the amidotransferase PabA. Biochemistry 1993;32:3763-68.
- Burg AW, Brown GM. The biosynthesis of folic acid. 8. Purification and properties of the enzyme that catalyzes the production of formate from carbon atom 8 of guanosine triphosphate. J Biol Chem 1968;243.
- 11. Nichols BP, Green JM. Cloning and sequencing of Escherichia coli ubiC and purification of chorismate lyase. J Bacteriol. 1992;174:5309-16.
- 12. Roiser JP, McLean A, Ogilvie AD, Blackwell AD, Bamber DJ, Goodyer I et al. The subjective and cognitive effects of acute phenylalanine and tyrosine depletion in patients recovered from depression. Neuropsychopharmacology 2005;30:775-85.
- 13. Harmer CJ, McTavish SF, Clark L, Goodwin GM, Cowen PJ. Tyrosine depletion attenuates dopamine function in healthy volunteers. Psychopharmacology (Berl) 2001;154:105-11.
- 14. Oli MW, Petschow BW, Buddington RK. Evaluation of fructooligosaccharide supplementation of oral electrolyte solutions for treatment of diarrhea: recovery of the intestinal bacteria. Digestive Diseases & Sciences. 1998;43(1):138-47.
- 15. Allen A, Cunliffe WJ, Pearson JP, et.al. Studies on gastrointestinal mucous. Scand J Gastroenterol 1984; s93:101-13.



- 16. van der Wiel-Korstanje JA, Winkler KC. The faecal flora in ulcerative colitis. J-Med-Microbiol. 1975;8:491-501.
- 17. Edwards CA.Duerden BI.Read NW. The effects of pH on colonic bacteria grown in continuous culture. Journal of Medical Microbiology.19(2):169-80, 1985.
- Caldarini MI, Pons S, D'Agostino D et al. Abnormal fecal flora in a patient with short bowel syndrome. An in vitro study on effect of pH on D-lactic acid production. Dig Dis Sci. 1996;41:1649-1652
- 19. Hove H, Mortensen PB. Colonic lactate metabolism and D-lactic acidosis. Dig Dis Sci 1995;40.
- 20. Shah M, Beuerlein M, Danayan K. An approach to the patient with a life-threatening acid-base disturbance: the acidemias. . University of Toronto Medical Journal 2001;78:122-28.
- 21. Uribarri J, Oh MS, Carroll HJ. D-lactic acidosis. A review of clinical presentation, biochemical features, and pathophysiologic mechanisms. Medicine (Baltimore) 30 1998;77:73-82.
- 22. Salvioli G, Salati R, Bondi M, et al. Bile acid transformation by the intestinal flora and cholesterol saturation in bile. Effects of Streptococcus faecium administration. Digestion. 1982;23:80-88.
- 23. Hirauchi K, Sakano T, Notsumoto S, Nagaoka T, Morimoto A. Measurement of K vitamins in food by high-performance liquid chromatography with fluorometric detection. Vitamins 1989;63:147-51.
- 24. E.A.Smith and G.T.Macfarlane. Studies on Amine Production in the Human Colon: Enumeration of Amine forming Bacteria and Physiological Effects of Carbohydrate and pH . Anaerobe 1996;2:285-97.
- 25. Mulligan JH.Snell EE. Transport and metabolism of vitamin B6 in lactic acid bacteria. Journal of Biological Chemistry. 252(3):835-9, 1977.
- 26. Buenrostro JL, Kratzer FH. Effect of Lactobacillus inoculation and antibiotic feeding of chickens on availability of dietary biotin. Poultry Science 1983;62:2022-29.
- 27. Zhang X, Rimpiläinen M, Simelyte E, Toivanen P. Characterisation of Eubacterium cell wall: peptidoglycan structure determines arthritogenicity. Ann Rheum Dis. 2001 Mar;60(3):269-74.
- 28. Kageyama A, Benno Y. Catenibacterium mitsuokai gen. nov., sp. nov., a gram-positive anaerobic bacterium isolated from human faeces. Int J Syst Evol Microbiol. 2000 Jul;50 Pt 4:1595-9.