# Lacolite Z, manufactured by Eskag Pharma Pvt. Ltd. Kolkata, India: The Next Generation Oral Rehydration Solution with Probiotics and Zinc for Comprehensive Gut and Immune Health

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## Abstract

Lacolite Z, an innovative oral rehydration solution (ORS) developed by Eskag Pharma, integrates probiotics and zinc with traditional electrolytes to offer a multifaceted approach to rehydration and immune support. This article reviews the key mechanisms through which probiotics and zinc contribute to gastrointestinal health, immune modulation, and overall wellbeing. The probiotics in Lacolite Z support gut health by competing against pathogens, producing antimicrobial substances, and enhancing the intestinal barrier, while zinc offers immune support, aids in intestinal repair, and promotes growth. This unique formulation makes Lacolite Z particularly effective for managing dehydration and diarrhea in both children and adults.

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#### I. Introduction

In human physiology, maintaining electrolyte balance is essential for a range of critical functions, including nerve signalling, muscle contraction, and fluid balance.<sup>1</sup> Electrolytes such as sodium, potassium, calcium, magnesium, and chloride play integral roles in maintaining these physiological processes.<sup>1</sup> However, factors like infections, gastrointestinal disorders, and dehydration can disrupt electrolyte levels, leading to potentially severe health consequences such as arrhythmias, muscle weakness, neurological symptoms, and even life-threatening complications.<sup>2</sup> Oral rehydration solutions (ORS) are commonly recommended to quickly replenish fluids and essential minerals lost through conditions like diarrhea, which remains a significant cause of morbidity and mortality worldwide, especially in developing countries.<sup>2</sup>

Traditional ORS solutions are designed to restore hydration and essential electrolytes but do not address the gut microbiome or immune function, both of which are crucial for overall recovery and health.<sup>2</sup> Emerging research in the field of gastroenterology and immunology highlights the importance of a healthy gut microbiome in managing gastrointestinal conditions and supporting systemic health. The gut's ecosystem, composed of beneficial bacteria, helps protect against pathogens, maintain the gut barrier, and modulate immune responses.<sup>2</sup> However, during episodes of diarrhea and dehydration, this delicate balance is often disrupted, making it beneficial to include gut-restoring agents in rehydration therapy.

Lacolite Z, developed by Eskag Pharma, is an innovative ORS product that combines traditional electrolytes with probiotics and zinc. This formulation provides not only rapid rehydration and electrolyte balance but also supports gut health and immune function, making it uniquely effective for comprehensive gastrointestinal care. The probiotics included in Lacolite Z- namely, *Bacillus coagulans, Lactobacillus acidophilus*, and *Lactobacillus rhamnosus*- are well-researched strains that help restore gut flora, compete against pathogens, and strengthen the intestinal barrier. Meanwhile, zinc plays an essential role in cellular repair and immune response, further aiding recovery from gastrointestinal distress. As a result, Lacolite Z is designed to be more than a standard ORS; it is a holistic rehydration solution that addresses multiple facets of recovery from dehydration and diarrhea. The combined action of probiotics, zinc, and electrolytes provides an integrated approach, potentially reducing the duration of diarrhea, supporting immune health, and promoting a faster return to well-being in both adults and children.

## Importance and Clinical Implications of Electrolyte Balance in Physiological Functions

Oral rehydration solution (ORS) or electrolytes are essential for fundamental physiological functions, including maintaining cellular electrical neutrality and enabling the generation and conduction of action potentials in nerves and muscles.<sup>1</sup> Key electrolytes include sodium, potassium, chloride, magnesium, calcium, phosphate, and bicarbonate, which are primarily derived from dietary intake and fluids. Electrolyte imbalances, whether

hyper- or hypo-concentrations, can disrupt normal bodily functions, potentially leading to severe and lifethreatening complications.<sup>1</sup> Sodium, an osmotically active extracellular cation, is a critical electrolyte for maintaining extracellular fluid volume and cellular membrane potential. Among electrolyte disorders, hyponatremia is the most prevalent and is defined by a serum sodium concentration below 135 mmol/L. Neurological symptoms of hyponatremia may include headache, confusion, nausea, and delirium. Conversely, hypernatremia, indicated by serum sodium levels exceeding 145 mmol/L, can manifest as tachypnoea, sleep disturbances, and agitation.<sup>2-4</sup> Potassium, primarily an intracellular cation, is essential for cellular function, particularly in cardiac rhythm regulation. Abnormal potassium levels can lead to arrhythmias; hypokalaemia, defined by serum potassium below 3.6 mmol/L, often presents with muscle weakness, fatigue, and fasciculations.<sup>5-</sup> <sup>8</sup> Calcium, a predominantly extracellular ion, is vital for neuromuscular function and blood clotting. Hypocalcaemia, which requires serum albumin correction for total calcium levels, is diagnosed when the corrected serum calcium is under 8.8 mg/dL, as seen in conditions like vitamin D deficiency and hypoparathyroidism.<sup>9-11</sup> Bicarbonate, regulated by the blood's acid-base status, can become imbalanced through bicarbonate loss, such as in chronic diarrhoea, leading to metabolic acidosis.<sup>12-13</sup> Magnesium, an intracellular cation, plays a role in enzymatic function and neuromuscular activity. Hypomagnesemia is diagnosed when serum magnesium falls below 1.46 mg/dL.<sup>14-15</sup> Chloride, a major extracellular anion, may increase in hyperchloremia, often secondary to gastrointestinal bicarbonate loss.<sup>16</sup> Phosphorus, present as phosphate in extracellular fluid, is prone to imbalance due to factors such as inadequate dietary intake, gastrointestinal malabsorption, or altered renal excretion.<sup>17</sup>

# Advances in the Management of Diarrhoeal Illnesses: ORS, Probiotics, and Zinc Supplementation

Diarrhoea or dysentery due to infectious gastroenteritis or any other conditions remain significant global causes of hospital admissions, mortality, and morbidity. The primary treatment for these conditions is the replacement of water and electrolyte losses via ORS.<sup>1</sup> However, as ORS does not directly decrease stool volume, nor does it reduce the duration or severity of diarrhoea, recent research has shifted towards exploring the molecular mechanisms of intestinal pathogens and evaluating alternative therapeutic strategies. Probiotics, defined as non-pathogenic microorganisms, withstand digestive processes to reach the colon in a viable state, where they positively influence host health.<sup>18-19</sup> Over recent decades, research publications on the role of intestinal microflora and the therapeutic impact of probiotics, particularly in acute infectious gastroenteritis, have significantly increased.<sup>19</sup> Additionally, the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) recommend zinc supplementation during diarrheal episodes in developing countries.<sup>20-21</sup> Probiotics are also recommended by the WHO in diarrhoea. This recommendation is based on numerous trials indicating that zinc (in forms such as zinc acetate, gluconate, or sulphate, either alone or combined with other vitamins, micronutrients, or ORS) effectively reduces the duration, severity, and recurrence of diarrheal episodes.<sup>20-21</sup>

While standard electrolyte formulations typically contain essential minerals like sodium, potassium, chloride, calcium, magnesium, bicarbonate, and phosphate, *Lacolite Z*, produced by **Eskag Pharma Pvt. Ltd.**, offers distinct additional components: **Oral Rehydration Salts (ORS)** combined with **Probiotics** and **Zinc**. ORS helps quickly restore hydration and electrolyte balance, particularly effective in preventing dehydration during acute diarrheal conditions. Probiotics supports gut health by replenishing beneficial bacteria, aiding in digestion, and improving gut barrier function, especially important when electrolyte imbalance is due to gastrointestinal issues. Zinc Plays a crucial role in immune function and aids in the recovery from infections, with particular efficacy in reducing the duration and severity of diarrhoeal episodes.

# Special Features of Laccolite Z manufactured by Eskag Pharma Pvt. Ltd., Kolkata, India

Lacolite Z by Eskag Pharma Pvt. Ltd., Kolkata, India stands out as an advanced oral rehydration solution (ORS) formulated to address dehydration and electrolyte imbalance with additional health benefits. Lacolite Z - is a 3 in 1 comprehensive therapy for diarrhoea and dehydration. Lacolite Z has Probiotics and Zinc in addition to ORS. Each 4.6 gm sachet contains a low-osmolarity ORS blend with essential electrolytes-sodium chloride, potassium chloride, and sodium citrate-for optimal fluid and electrolyte replenishment. The inclusion of dextrose aids in efficient absorption of electrolytes and fluids, enhancing the rehydration process. A unique feature of Lacolite Z is the addition of zinc sulphate monohydrate, providing 4 mg of elemental zinc, which supports immune health and reduces both the duration and recurrence of diarrheal episodes, as recommended by the World Health Organization (WHO). Furthermore, Lacolite Z contains probiotics- *Bacillus coagulans* (0.5 billion cells), *Lactobacillus acidophilus* (0.25 billion cells), and *Lactobacillus rhamnosus* (0.25 billion cells)- which contribute to gut health by restoring healthy intestinal microflora, thereby enhancing recovery from gastroenteritis and other digestive disturbances. Packaged in convenient 4.6 gm sachets for easy administration, Lacolite Z serves as a comprehensive rehydration solution, targeting hydration, immune support, and digestive health, especially beneficial in paediatric and adult populations susceptible to dehydration and electrolyte loss.

# Mechanisms of action of Probiotics present in Lacolite Z

The mechanisms of action of probiotics in Lacolite Z, specifically Bacillus coagulans, Lactobacillus acidophilus, and Lactobacillus rhamnosus, involve multiple, distinct pathways that enhance gastrointestinal health and immune function. These probiotics exhibit direct antagonism against pathogenic microorganisms by competing for adhesion sites on the intestinal epithelium. This competitive inhibition prevents pathogens from adhering and colonizing the gut, thus reducing the risk of infection.<sup>22</sup> Probiotic strains such as Lactobacillus acidophilus and Bacillus coagulans produce antimicrobial compounds, including bacteriocins and organic acids, which create a hostile environment for pathogenic bacteria. This production not only lowers the pH of the intestinal lumen but also contributes to pathogen inhibition.<sup>23</sup> Probiotics strengthen the integrity of the gut epithelial barrier by promoting the expression of tight junction proteins. This action decreases intestinal permeability, thereby limiting the translocation of pathogens and toxins into the bloodstream and reducing systemic inflammation.<sup>24</sup> The probiotics in Lacolite Z interact with immune cells and receptors, particularly Tolllike receptors (TLRs). This interaction stimulates both the innate and adaptive immune responses, leading to increased activation of macrophages and natural killer cells, and enhancing the production of immunoglobulins and specific cytokines, such as IL-10 and TGF-β, which are crucial for immune regulation.<sup>25</sup> Probiotics promote the development of gut-associated lymphoid tissue (GALT), thereby increasing the production of immunoglobulin A (IgA) and enhancing local mucosal immunity. This is particularly beneficial in managing gastrointestinal infections.<sup>26</sup> Certain strains, particularly *Lactobacillus rhamnosus*, can modulate the inflammatory response by decreasing the production of pro-inflammatory cytokines (e.g., TNF-a, IL-6) while promoting anti-inflammatory cytokines. This balance helps alleviate symptoms associated with gastrointestinal inflammation.<sup>27</sup> The presence of different probiotic strains in Lacolite Z may exhibit synergistic effects, enhancing their adherence and efficacy during episodes of diarrhea. For example, the presence of *Lactobacillus casei GG* can improve the adherence of Bifidobacterium bifidum, thereby enhancing their overall effectiveness in promoting gut health.<sup>28</sup> Probiotics present in the formulation may further contribute to gut health by counteracting bacterial toxins, influencing inflammatory pathways, and improving the maturation of brush border enzymes and glucose carriers in the enterocyte membrane.<sup>29</sup> These multifaceted mechanisms underscore the therapeutic potential of the probiotics in Lacolite Z for managing gastrointestinal disorders and supporting overall gut health.

# Mechanisms of action of Zinc present in Lacolite Z

Zinc is a vital micronutrient involved in metabolism and immune functions. It reduces ion secretion and nitric oxide production, supporting gut homeostasis and mucosal function.<sup>30</sup> Zinc enhances appetite, absorption, enterocyte regeneration, and intestinal barrier maintenance, restoring enzyme activity and intestinal permeability.<sup>31</sup> It supports humoral and cellular immunity by boosting lymphocyte proliferation and specific immunoglobulin production, such as those targeting invasion plasmid-encoded antigens.<sup>32</sup> In vitro, zinc promotes ion absorption and counters the effects of heat-labile enterotoxin from Vibrio cholerae via intracellular cAMP but not heat-stable E. coli enterotoxin.<sup>33</sup> It protects enterocytes from E. coli-induced damage by preserving membrane integrity, reducing bacterial adhesion, blocking invasiveness, and regulating cytokine production like IL-8 and TNF- $\alpha$  while enhancing transforming growth factor- $\beta$  synthesis.<sup>34</sup> Zinc also supports growth and weight gain through effects on growth hormone and insulin-like growth factor-1.<sup>35</sup> In children with shigellosis, 20 mg of elemental zinc for 2 weeks significantly increases lymphocyte proliferation and immunoglobulin production, restoring immune function.<sup>36</sup> Overall, zinc is critical for intestinal health, immune enhancement, and recovery from stress or infection.

# Why Choose Lacolite Z Over Standard ORS?

Lacolite Z offers distinct advantages over traditional oral rehydration solutions (ORS), providing a more comprehensive approach to managing dehydration and supporting overall gut and immune health. While standard ORS products are effective for basic rehydration by replenishing lost fluids and electrolytes, they do not address the broader needs of gastrointestinal health and immune function, particularly during or after episodes of diarrhea. Lacolite Z addresses these gaps, making it a superior choice for patients seeking a complete solution.

# Enhanced Gut Health with Probiotics:

Unlike standard ORS, Lacolite Z contains carefully selected probiotic strains, including Bacillus coagulans, Lactobacillus acidophilus, and Lactobacillus rhamnosus. These probiotics work by restoring the gut's natural flora, which is often disrupted during diarrhea or gastrointestinal illness. They help to prevent the colonization of harmful bacteria, support digestion, and strengthen the intestinal barrier. By promoting a healthy microbiome, Lacolite Z not only addresses immediate hydration needs but also supports long-term gut health.

# Immune System Support Through Zinc Supplementation:

Zinc is a vital micronutrient with proven benefits for immune health and intestinal recovery, yet it is not present in standard ORS solutions. Lacolite Z includes zinc sulfate, which has been shown to reduce the duration and severity of diarrheal episodes. Zinc supports immune response by enhancing lymphocyte function and promoting the production of antibodies, helping the body more effectively fight infections and recover faster. This is especially valuable for children and individuals with compromised immunity.

# Reduced Inflammation and Faster Recovery:

The probiotics in Lacolite Z not only help restore gut flora but also have anti-inflammatory properties that can alleviate gastrointestinal symptoms. For example, Lactobacillus rhamnosus can reduce the production of pro-inflammatory cytokines, helping to relieve inflammation in the gut. This effect is particularly beneficial for patients experiencing discomfort or irritation due to gastrointestinal distress, allowing for a faster and more comfortable recovery.

# Protection Against Future Episodes of Diarrhoea:

Probiotics and zinc together create a resilient gut environment that can offer protection against future episodes of diarrhea. The probiotics promote a balanced microbiome, which is critical for preventing the growth of harmful bacteria, while zinc strengthens the gut barrier and immune defenses. Regular use of Lacolite Z may thus reduce the risk of recurrent gastrointestinal issues, supporting longer-term gut health and overall wellbeing.

## Ideal for a Wide Range of Patients:

Lacolite Z's unique formulation makes it suitable for various patient groups, including children, adults, and the elderly. Its gentle but effective combination of ORS, probiotics, and zinc is designed to meet the diverse needs of individuals prone to dehydration, whether due to age, illness, or other health conditions. The product is also ideal for individuals traveling to areas where diarrheal illnesses are prevalent, offering preventative support in addition to rehydration.

## Convenient and Easy to Use:

Packaged in convenient sachets, Lacolite Z is easy to prepare and administer. This makes it ideal for home use, travel, and even clinical settings, providing an all-in-one solution for dehydration, gut health, and immune support. Its portability and ease of use make it practical for on-the-go rehydration, particularly for those in vulnerable health situations.

# II. Conclusion

Lacolite Z represents an advanced approach to rehydration therapy, going beyond conventional ORS by integrating probiotics and zinc. This formulation not only replenishes fluids and electrolytes but also provides significant benefits for gut health and immune function. Probiotics in Lacolite Z help to restore microbial balance and reduce inflammation in the gut, while zinc contributes to intestinal repair and immune resilience. As a result, Lacolite Z is well-suited for patients experiencing dehydration from gastrointestinal disturbances, offering a more comprehensive solution to electrolyte management and gut health.

#### References

- [1] Ferrannini E. Sodium-Glucose Co-transporters and Their Inhibition: Clinical Physiology. Cell Metab. 2017 Jul 05;26(1):27-38.
- Palmer LG, Schnermann J. Integrated control of Na transport along the nephron. Clin J Am Soc Nephrol. 2015 Apr 07;10(4):676-87.
  Buffington MA, Abreo K. Hyponatremia: A Review. J Intensive Care Med. 2016 May;31(4):223-36.
- [4] Ambati R, Kho LK, Prentice D, Thompson A. Osmotic demyelination syndrome: novel risk factors and proposed pathophysiology. Intern Med J. 2023 Jul;53(7):1154-1162.
- [5] Gumz ML, Rabinowitz L, Wingo CS. An Integrated View of Potassium Homeostasis. N Engl J Med. 2015 Jul 02;373(1):60-72.
- [6] Ellison DH, Terker AS, Gamba G. Potassium and Its Discontents: New Insight, New Treatments. J Am Soc Nephrol. 2016 Apr;27(4):981-9.
- [7] Stedwell RE, Allen KM, Binder LS. Hypokalemic paralyses: a review of the etiologies, pathophysiology, presentation, and therapy. Am J Emerg Med. 1992 Mar;10(2):143-8.
- [8] Viera AJ, Wouk N. Potassium Disorders: Hypokalemia and Hyperkalemia. Am Fam Physician. 2015 Sep 15;92(6):487-95.
- [9] Veldurthy V, Wei R, Oz L, Dhawan P, Jeon YH, Christakos S. Vitamin D, calcium homeostasis and aging. Bone Res. 2016;4:16041.
- [10] Cooper MS, Gittoes NJ. Diagnosis and management of hypocalcaemia. BMJ. 2008 Jun 07;336(7656):1298-302.
- [11] Turner JJO. Hypercalcaemia presentation and management . Clin Med (Lond). 2017 Jun;17(3):270-273.
- [12] Hamm LL, Nakhoul N, Hering-Smith KS. Acid-Base Homeostasis. Clin J Am Soc Nephrol. 2015 Dec 07;10(12):2232-42.
- [13] Kraut JA, Madias NE. Adverse Effects of the Metabolic Acidosis of Chronic Kidney Disease. Adv Chronic Kidney Dis. 2017 Sep;24(5):289-297.
- [14] Jahnen-Dechent W, Ketteler M. Magnesium basics. Clin Kidney J. 2012 Feb;5(Suppl 1):i3-i14.
- [15] Hansen BA, Bruserud Ø. Hypomagnesemia as a potentially life-threatening adverse effect of omeprazole. Oxf Med Case Reports. 2016 Jul;2016(7):147-9.
- [16] Morrison G. Serum Chloride. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd ed. Butterworths; Boston: 1990.

- [17] Berkelhammer C, Bear RA. A clinical approach to common electrolyte problems: 3. Hypophosphatemia. Can Med Assoc J. 1984 Jan 01;130(1):17-23.
- [18] World Health Organization. (2005). The Treatment of Diarrhea: A Manual for Physicians and Other Senior Health Workers. WHO Press, Geneva.
- [19] Guandalini, S. (2011). "Probiotics for prevention and treatment of diarrhea." Journal of Clinical Gastroenterology, 45, S149-S153.
- [20] Bhutta, Z. A., et al. (2000). "Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials." Journal of Pediatrics, 135(6), 689-697.
- [21] WHO & UNICEF. (2004). Clinical management of acute diarrhoea: WHO/UNICEF Joint Statement. WHO/UNICEF, Geneva/New York.
- [22] Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118. Proc Natl Acad Sci U S A. 2007 Aug 28;104(18):7617-21. doi: 10.1073/pnas.0700440104. PMID: 17456596.
- [23] Reid G, Jass J, Sebulsky MT, McCormick JK. Potential uses of probiotics in clinical practice. Clin Microbiol Rev. 2003 Oct;16(4):658-72. doi: 10.1128/CMR.16.4.658-672.2003. PMID: 14557292.
- [24] Bron PA, van Baarlen P, Kleerebezem M. Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. Nat Rev Microbiol. 2012 May 21;10(1):66-78. doi: 10.1038/nrmicro2713. PMID: 22101920.
- [25] Foligne B, Nutten S, Grangette C, Dennin V, Goudercourt D, Poiret S, et al. Correlation between in vitro and in vivo immunomodulatory properties of lactic acid bacteria. World J Gastroenterol. 2007 Dec 14;13(2):236-43. doi: 10.3748/wjg.v13.i2.236. PMID: 17206761.
- [26] Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol. 2004 Jun;4(6):478-85. doi: 10.1038/nri1373. PMID: 15173836.
- [27] O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, et al. Lactobacillus and Bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology. 2005 Feb;128(3):541-51. doi: 10.1053/j.gastro.2004.11.050. PMID: 15765388.
- [28] Chapman CM, Gibson GR, Rowland I. Health benefits of probiotics: are mixtures more effective than single strains? Eur J Nutr. 2011 Jun;50(1):1-17. doi: 10.1007/s00394-010-0166-z. PMID: 20571891.
- [29] McFarland LV. Systematic review and meta-analysis of Saccharomyces boulardii in adult patients. World J Gastroenterol. 2010 Mar 14;16(18):2202-22. doi: 10.3748/wjg.v16.i18.2202. PMID: 20458757.
- [30] Rodriguez P, Darmon N, Chappuis P, Candalh C, Blaton MA, Bouchaud C, et al. Intestinal paracellular permeability during malnutrition in guinea pigs: effect of high dietary zinc. Gut. 1996 May;39(5):416-22. doi: 10.1136/gut.39.3.416. PMID: 8796238.
- [31] Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr. 1998 Aug;68(2 Suppl):447S-463S. doi: 10.1093/ajcn/68.2.447S. PMID: 9701160.
- [32] Rink L, Gabriel P. Zinc and the immune system. Proc Nutr Soc. 2000 Aug;59(4):541-52. doi: 10.1017/s0029665100000781. PMID: 11115789.
- [33] Hoque KM, Rajendran VM, Binder HJ. Zinc inhibits cAMP-stimulated Cl- secretion via basolateral K+ channel blockade in rat ileum. Am J Physiol Gastrointest Liver Physiol. 2005 Oct;289(4). doi: 10.1152/ajpgi.00026.2005. PMID: 15831804.
- [34] Crane JK, Naeher TM, Shulgina I, Zhu C, Boedeker EC. Effect of zinc in enteropathogenic Escherichia coli infection. Infect Immun. 2007 Mar;75(3):597-605. doi: 10.1128/IAI.00715-06. PMID: 17101652.
- [35] Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2002 Jun;75(6):1062-71. doi: 10.1093/ajcn/75.6.1062. PMID: 12036814.
- [36] Rahman MM, Vermund SH, Wahed MA, Fuchs GJ, Baqui AH, Alvarez JO. Simultaneous zinc and vitamin A supplementation in Bangladeshi children: randomized double blind controlled trial. BMJ. 2001 Feb 10;322(7283):289-93. doi: 10.1136/bmj.322.7283.289. PMID: 11157526.