

Is There Normal Blood Flora?

E Kalfin

Citation

E Kalfin. *Is There Normal Blood Flora?*. The Internet Journal of Microbiology. 2006 Volume 3 Number 1.

Abstract

Unknown microorganisms, which live as normal flora in every human's blood, were isolated in pure cultures and represented on microscope color microphotographs and electron microscope photographs in the blood of medical professors and associated professors. The aim of this article is to demonstrate 3 methods used as routine procedure in order to isolate normal blood flora in pure cultures.

INTRODUCTION

Indisputably, DNA-analyses are the most important discovery for microbiology (1, 2).

The imperative condition that each newly discovered microorganism should pass over in silence unless DNA-analysis is not realized turns microbiology upside down, having in mind that tuberculosis, plague, cholera, etc. are found using only their isolation in pure cultures and not DNA-analyses.

Pure cultures are the alpha and omega of microbiology in the past, present, and future because they allow a comprehensive study on newly discovered microorganisms, promoting their further DNA-analyses in every laboratory.

MATERIAL AND METHODS

CLASSIC METHOD FOR ISOLATION OF NORMAL BLOOD FLORA

LIQUID MEDIUM

BBL® Brain Heart Infusion with 0.25% sodium citrate, 20 mg/L gentamicin and 50 mg/L chloramphenicol, is distributed in sterile test tubes, 4.5 ml in each, and is sterilized in autoclave at 121°C, for 15 minutes. 0.5 ml venous human blood is inoculated in a tube and cultured for 30 days, at 37°C. After centrifugation 5 min/3000 rpm, a Gram's stained slide and a subculture sample on sheep blood agar (as contamination control sample) are prepared.

HUMAN BLOOD AGAR

Brain Heart Infusion with 1.2% Noble agar is sterilized at 121°C for 15 min, cooled at 50°C, mixed with 10% outdated human blood for transfusion and poured in plates. Positive blood culture sediment is inoculated evenly on the surface.

ACCELERATE METHOD FOR ISOLATION OF NORMAL BLOOD FLORA

The inoculated tubes are cultured at 43°C for 14 days instead at 37°C for 30 days and treated as described above.

RAPID METHOD FOR ISOLATION OF NORMAL BLOOD FLORA WITH VITAMIN K3, OR VITAMIN K1, 1G/L

0.2 ml blood from a BD Vacutainer® K2E 5.4 mg 3 ml should be taken and 0.4 ml sterile bidistilled water must be added. The mixture is swayed to help erythrocyte hemolysis. After 30 minutes stay at room temperature the hemolytic erythrocytes are cultured in a test tube with 2 ml Brain Heart Infusion BBL® which contains also 1 mg/ml vitamin K3, or vitamin K1 as growth factor, at 43°C, for 3 days and treated as describe above.

RESULTS

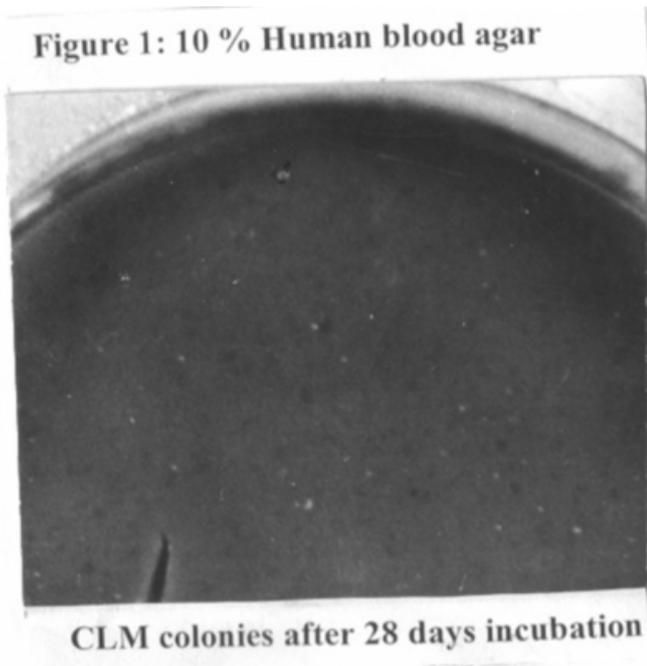
CLASSIC METHOD FOR ISOLATION OF NORMAL BLOOD FLORA

Using this method color pictures and electron microscope pictures were demonstrated in another articles (3, 4). The microorganisms were observed as spherical bodies inside human erythrocytes, situated as in nests. These unique nests are specific for normal blood flora microorganisms.

CULTURE PROPERTIES ON HUMAN BLOOD AGAR:

After 28 days, normal blood microorganisms formed tiny gray colonies, which were almost invisible with a naked eye. The colonies were scanty. They were spherical, soft and easily removable by a loop.

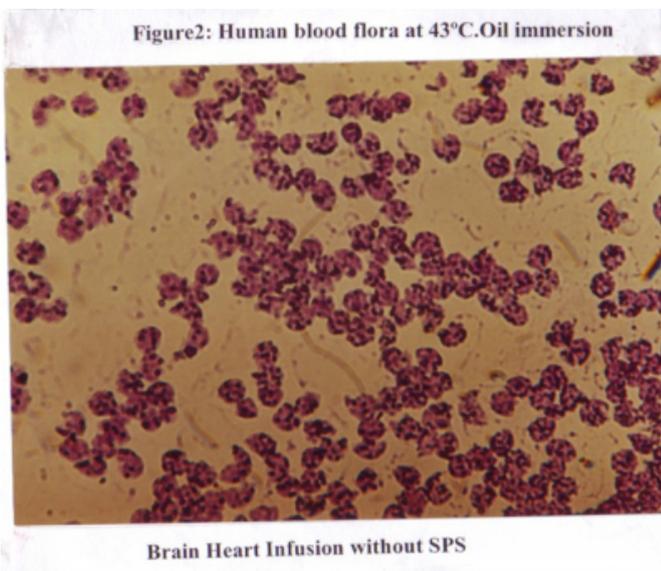
Figure 1



ACCELERATE METHOD FOR ISOLATION OF NORMAL BLOOD FLORA

At 43°C normal blood flora microorganisms are growing faster than at 37°C and the microorganisms can be seen inside the human erythrocytes as in nests after 14 days only.

Figure 2

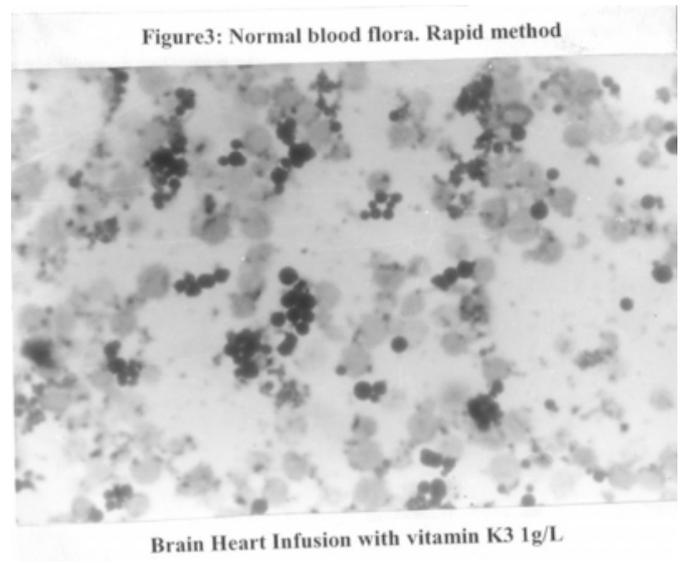


RAPID METHOD FOR ISOLATION OF NORMAL BLOOD FLORA

Vitamin K3 or vitamin K1, 1 g/L, are a powerful growth factor for human blood normal flora. If the erythrocytes are destroyed with distilled water the microorganisms multiply

in the medium as yeast-like microorganisms faster than inside the erythrocytes

Figure 3



DISCUSSION

Only 0.2 ml human blood is necessary to isolate a pure culture of normal blood flora for 3 days, in every laboratory of the world, and therefore, hundreds of researchers to realize DNA-analyses of the unknown up to the present moment microorganisms. Thus they would finally start solving the problem of blood transfusion, containing donor's normal blood flora (5).

CONCLUSIONS

Practically, the sterilization of the normal flora in transfused blood is impossible on a large scale at present and in near future. The human being is a hyper organism, comprising only 10% human cells and 90% bacteria, viral, and fungal cells. (6) This fact makes adequate the hypothesis that transfusions should not require blood sterilization, but a similarity between donor's and recipient's blood. It is illogical for the human being to postulate sterility while the nature has created normal flora in blood.

The consequences of the mixing of microorganism and their genetic materials in different individuals, as well as the significance of normal blood flora for medicine as a whole, go beyond the author's knowledge and the author is not going to discuss them.

ACKNOWLEDGMENT

The author would like to thank to Assoc. Prof. M. Markova Chief Biochemical Laboratory at the State University

Hospital of Pulmonary Disease Sofia, Bulgaria, for blood supplies

CORRESPONDENCE TO

Dr Emil Kalfin 2 Grivitza Str Sofia 1202, Bulgaria
Telephone and Fax: + 35928315686 Email:
dr_emil_kalfin@yahoo.com

References

1. Nikkary Mc Laughlin, W. Ri, Deborah E. Dodges, and David A. Relman 2001 Dose blood of healthy subjects contain bacterial ribosomal DNA? *J.Clin. Microbiol.* 39:1956-1859
2. Richard W. McLaughlin, Hojatolla Vali, Peter C.K,

- Rogers G. E. Palfree, Angela De Ciccio, Marc Siros, Darakhshan Ahmad, Richard Villemur, Marcel Desresiers and Eddie C.S. Chan 2002 Are There Naturally Occuring Pleomorphic Bacteria in the Blood of Healthy Humans? *J.Clin Microbiol.* 40. 12. 4771-4775
3. Kalfin E 1997-1998 Resident microbial flora in human erythrocytes *Journal of Culture Collections* 2,77-82 DOAJ, Bioline International
4. Kalfin E The blood of a healthy person is never sterile because human blood has normal flora www.medicalbg.com 2004- 04-05 English Version
5. Kalfin E Chlamydia-like Microorganisms Live in Donor's Blood as Normal Flora *The Internet Journal of Internal Medicine* 2005 Volume 5, Number 2, DOAJ
6. Jeremy K Nicholson, Elaine Holms, John C. Linden, and Jan D. Wilson. The challenges of modeling mammalian biocomplexity *Nature Biotechnology* 22.1268-127/ 2004

Author Information

E. Kalfin, M.D, Ph.D.

Laboratory of Microbiology, State University Hospital of Pulmonary Diseases