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**THE NATIONAL INSTITUTE OF RESEARCH-DEVELOPMENT
FOR MICROBIOLOGY AND IMMUNOLOGY
"CANTACUZINO"**

TESTING OF THE MULTISTAGE WATER FILTER
Manufactured by SC BLUE STAR INTERNATIONAL
Bucharest, Sector 6

The following collectives collaborated to the microbiological and parasitology tests, in accordance with the attached results:

- The Laboratory of Microbial Cultures – tested 14 microorganisms
  11 bacteria 3 fungi
  Ph.D Marilena Dinulescu

- The National Reference Center for Brucelose, Listeriose and Anthrax - tested B. Anthracis
  Ph.D Dana Magdalena Caplan

- The Laboratory of Vibrio (CNR cholerae) – tested V. Cholerae
  Ph.D Anca Israil

- The Laboratory of Parasitology - tests on 7 parasites
  Lecturer, Ph.D Dan Stariu

General Manager

Professor Marian Negut, Ph.D

THE LABORATORY OF MICROBIAL CULTURES

TESTING OF MULTISTAGE WATER FILTER EFFICIENCY

14 microbial cultures were tested – 11 bacterial cultures (cocci gram + bacilli gram) and 3 fungal cultures (levuris and filamentous fungi): *Candida albicans, Geotrichum candidum and Aspergillus flavus.*

The test stages were:

- The preparation of the filtrate suspension by revitalizing a test tube of lyophilized microorganism and by further inoculation of each tested microorganism in the culture media.
- The control of each tested culture purity.
- The determination of the number of germs in the filtrate suspension and the carrying out of successive dilutions out of which the gelose medium was further inoculated with.
- The connection of the filter and of the filtering device (previously sterilized) to the vacuum pump.
- The determination of the number of germs after filtration in each dilution, by inoculating the colonies grown on the 3 Petri dishes (3 Petri dishes with gelose were used for each dilution, to get an average).
- The incubation in the thermostat for 24-48 h (depending on the growth titration of each species) at 37° C, in order to develop the colonies which were to be numbered.
- The calculation of the filter efficiency by a formula given by the beneficiary of our services.

Materials and Methods

14 lyophilized microorganisms were used, of which 5 from the international collections (*Proteus mirabilis* NCTC 10416, *Pseudomonas aeruginosa* NCTC 13189, *E. coli* ATCC 25922, *Salmonelle typhi* NCTC 10735, *Staphylococcus aureus* ATCC 25923) and one from the collection of the Pasteur Institute –Paris- *Klebsiella pneumoniae* IP 12349. The other tested strains were from the microbial collection of the Cantacuzino Institute (see Table 1).

Microscopic examinations were carried out on each strain, after its revitalization, to see the purity of the cultures which were to be used for tests (microscopic preparations, Gram colored, from the 24 h cultures on gelose).

After had prepared the initial suspension (from 24 h cultures) with a concentration similar to the standard no. 1 Mc. Farland, successive dilutions (1/10) were made with physiological solution.

0.1 ml from each dilution were inoculated on each Petri dish (Ø 10 cm), by using 3 Petri dishes for each dilution.

The preparation of the sterile materials, the media (gelose and Sabouraud) distribution in test tubes and Petri dishes were made by classical microbiological methods.

After filtration, successive dilutions of 1/10 were made from the filtered suspension by using the same methodology as the one used before the filtration.

Further, all the dishes inoculated with non-filtered and filtered suspensions were incubated at 37° C for 24-48 h.

The number of the colonies grown after the incubation at 37° C was further found and the averages for the dilutions were made for both before and after the filtration suspensions.

The formula given by the beneficiary to find the efficiency was applied for each tested strain:

$$\text{The filtration efficiency} = \frac{\text{No. of microorganisms trapped by the filter}}{\text{No. of microorganisms in the initial suspension}} \times 100$$

$$= \frac{\text{(No. of introduced germs – No. of filtered germs)}}{\text{No. of introduced germs}} \times 100$$

Thus, the efficiency is expressed in percentages (see Table 1).

Results

The obtained results are shown in the Table no. 1, percentages over 99.99999 as regards the bacteria and between 99.125 – 99.98 as regards the fungi being noticed.

Conclusions

1. **The highest efficiency has been obtained with *Pseudomonas aeruginosa*, followed by *Shigella flexneri, Salmonella Typhi, Proteus mirabilis* and *E. Coli.***
2. **A relatively lower efficiency (among the bacteria) can be noticed with *Bacillus subtilis* (99.94 %) and *Staphilococccus epidermitis* (99.91 %).**
3. **Even with fungi the efficiency is over 99 % - for instance *Geotrichum candidum* – 99.998 %.**
4. **The efficiency is over 99 % with all those 14 tested strains.**

The Experts Team

(see 3th page)

Table 1

Tested microorganisms and the filtration efficiency

No.	*Microorganisms*	*Non-filtered*	*Filtered*	*Eficiency %*
1.	***B*** - Pseudomonas aeruginosa NCTC-13189 – Bacil piocianic	$9,8 \times 10^{14}$	$2,5 \times 10^{7}$	99.99999
2.	***B*** - Shigella flexneri IC - 12633	$5,20 \times 10^{12}$	$4,6 \times 10^{5}$	99.99999
3.	***B*** - Salmonella typhi IC - 10872	$1,47 \times 10^{12}$	$1,26 \times 10^{6}$	99.99992
4.	***B*** - Proteus mirabilis NCTC - 10416	$3,03 \times 10^{12}$	$3,62 \times 10^{6}$	99.9999
5.	***B*** - Escherichia coli ATCC - 25922	$2,29 \times 10^{12}$	$3,8 \times 10^{6}$	99.9999
6.	***B*** - Enterobacter cloaccae IC-13482	$6,70 \times 10^{9}$	$2,41 \times 10^{4}$	99.99965
7.	***B*** - Staphylococcus aureus ATCC-25923	$1,3 \times 10^{12}$	$1,10 \times 10^{7}$	99.99916
8.	***F*** - Geotrichum candidum IMF-126	$1,24 \times 10^{5}$	$3 \times 10^{\circ}$	99.998
9.	***F*** - Candida albicans IC - 130	$1,10 \times 10^{6}$	$2,4 \times 10^{2}$	99.98
10.	***B*** - Klebsiella pneumoniae IP-12349	$4,70 \times 10^{10}$	$2,16 \times 10^{7}$	99.96
11.	***B*** - Bacillus subtilis IC - 13358	$2,47 \times 10^{7}$	$1,53 \times 10^{4}$	99.94
12.	***B*** - Staphylococcus epidermitis IC-13230	$4,5 \times 10^{10}$	$4,15 \times 10^{7}$	99.91
13.	***B*** - Salmonella enteritidis IC-10872	$1,33 \times 10^{11}$	$3,1 \times 10^{8}$	99.80
14.	***F*** - Aspergillus flavus IC-107	8×10^{5}	7×10^{3}	99.125

Head of Team
Microbial Collection
Ph. d. Ana Vladescu

Tested by :
Ph. d. Marilena Dinulescu
Scientific Researcher, degree I

General Manager
Professor Marian Negut, Ph. D.M.D

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Bucharest, September 17, 2003
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BACTERIOLOGICAL TEST BULLETIN

Testing of the bacterial filtration efficiency of the "Multistage Water Filter" made by S.C. BLUE STAR INTERNATIONAL S.R.L.

The bacterial filtration efficiency of the "Multistage Water Filter" was tested by filtering a solution of bacterial culture, 10^{20} anthrax germs/liter, using a special device which ensured the bacterial culture flow through the filter at a pressure of 0.5 atm.

The filtration flow rate was of about 600 ml.

The samples of the initial and the filtered solutions were analyzed by making different dilutions which were inoculated on Petri dishes with culture media. After a 24 hour incubation at 37^0 C, the formed colonies were numbered. The results of the bacteriological test have made evident, in the case of B. anthracis, a filtration efficiency as follows:

Sample	Bacterial concentration no. of B. anthracis/l	Filtration efficiency (%)
Non-filtered suspension of B. anthracis culture	57.10^{20}	
Filtrate of bacterial suspension	51.10^4	99.99999999999999

General Manager
Prof. M. Negut Ph.D

INSTITUTUL NAȚIONAL DE CERCETARE DEZVOLTARE PENTRU MICROBIOLOGIE ȘI IMUNOLOGIE CANTACUZINO MINISTERUL SĂNĂTĂȚII ROMÂNIA

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Ph. D Dana Magdalena Cõplõn

THE MINISTRY OF HEALTH
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The Vibrio Laboratory

Bucharest, 10.09.2003
Registration No:

Assessment of filtration efficiency of the "MULTISTAGE WATER FILTER" created by SC Blue Star International SRL

Method:

An indigenous ***Vibrio cholerae*** biotype eltor serotype Ogawa was inoculated in six tubes on the surface of slant agar 2%.

After18-24 h incubation at 37^0 C, from these cultures a bacterial suspension in 1000 ml sterile physiological solution was prepared and adjusted to the concentration corresponding to Mc Farland standard of 10 I.U.

0.1 ml of this suspension (concentration 10 I.U. McF) was used to prepare dilutions from $10^{-1} \rightarrow 10^{-10}$ by help a sterile physiological solution.

0.1 ml of each above-mentioned dilutions were inoculated in a Petri dish on the surface of 2% agar and thereafter all dishes were inoculated 18-20 h at 37^0 C.

The initial bacterial suspension of 10 I.U. Mc Farland concentration was passed through the respective filter.

From the obtained filtrated product , dilutions of $10^{-1} \longrightarrow 10^{-10}$ in sterile physiological solution were prepared and thereafter 0.1 ml of each dilution were inoculated in a Petri dish on 2% agar.

All dishes were inoculated 18-20 h at 37^0 C.

Results:

After the inocubation period, all Petri dishes were examined by comparison, concerning the number of microbial colonies.

The corresponding formula to estimate the filtration efficiency was applied considering the number of CFU (colony forming units) in dilutions of the initial bacterial suspension *versus* the number of the CFU in dilutions of the filtrated product.

Dilution	Initial bacterial suspension UFC	Filtered product UFC	Filtration efficiency %
10^{-5}	198	0	100
10^{-6}	97	0	100
10^{-7}	60	0	100

Conclusion:

The filter proved to exhibit efficiency by retaining 100 % ***Vibrio cholerae*** bacterial cells (on special condition of preserving its physical integrity).

General Director
Professor Marian Negut, M.D.

Chief of Vibrio Laboratory
M.D. Anca Israil

THE LABORATORY OF PARASITOLOGY

REPORT ON THE TESTS CARRIED OUT WITH THE "MULTISTAGE FILTER" PRESENTED BY S.C. BLUE STAR INTERNATIONAL S.R.L.

The tests were focused on the filter efficiency in trapping protozoa cysts and helminth eggs that could be found in the drinking water. The tested germs can cause hydric epidemics.

The filtering capacity was tested by using the following germs:

- *Giardia duodenalis* cysts (8-14/6-10 μm) in a density of $1x10^5$/ml
- *Entamoeba histolytica* cysts (10-15 μm) in a density of $1x10^4$/ml
- *Cryptosporidium parvum* oocysts (< 4 μm/ml) in a density of $1x10^6$/ml
- *Ascaris lumbricoudes* eggs (67-75/35-50 μm) in a density of $1x10^4$/ml
- *Trichuris trichiura* eggs (50-55/20-25 μm) in a density of $1x10^3$/ml
- *Enterobius vermicularis* eggs (50-52/20 μm) in a density of $1x10^4$/ml
- *Hymenolepis nana* eggs (30-50 μm) in a density of $1x10^4$/ml
- *Taenia solium* eggs (30-40 μm) in a density of $1x10^4$/ml

In the first stage, separate tests were carried out with each of the germs.

The control made on the final product, filtered and centrifuged, by direct examinations and samples of concentration, showed the following content of germs:

- *Giardia duodenalis* cysts - 0
- *Entamoeba histolytica* cysts - 0
- *Cryptosporidium parvum* oocysts - 0
- *Ascaris lumbricoudes* eggs - 0
- *Trichuris trichiura* eggs - 0
- *Enterobius vermicularis* eggs - 0
- *Hymenolepis nana* eggs - 0
- *Taenia solium* eggs – 0

In the second stage the filtration was made with a mixture of germs having an individual density of about ½ of the one used for the separate tests. **The results were identical, namely 0 germs in the filtered product.**

The filtration was repeated with five filters to eliminate any suspicion that could arise because of a possible non-uniformity of the product quality.

In all cases the results were similar, namely 0 germs in the filtered product.

Given the above mentioned ones, we consider the "Multistage Filter" sent to be tested by S.C. BLUE STAR INTERNATIONL S.R.L., efficient as regards the capacity to trap the parasitic germs accidentally present in the drinking water. Therefor, in our opinion, the filter can be manufactured and sold on the market.

Head of Laboratory
Lecturer Ph.D. Dan Steriu

TABLE 2

MICROORGANISMS TESTED AND THE EFFICIENCY OF THE FILTRATION

"MULTISTAGE WATER FILTER"

No.	*Microorganism (parasite)*	*Concentration of the initial solution germs/ml*	*Concentration of the filtrate germs/ml*	*Efficiency of the filtration %*
1.	*Giardia duodenalis cysts*	10^5	*0*	*100*
2.	*Entamoeba histolytica cysts*	10^4	*0*	*100*
3.	*Cryptosporidium parvum oocysts*	10^6	*0*	*100*
4.	*Ascaris lumbricoides eggs*	10^4	*0*	*100*
5.	*Trichuris trichiura eggs*	10^3	*0*	*100*
6.	*Enterobius vermicularis eggs*	10^4	*0*	*100*
7.	*Hymenolepis nana eggs*	10^4	*0*	*100*
8.	*Taenia solium eggs*	10^4	*0*	*100*

01.09.2003
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