United States Patent [19]

Abdou et al.

[11] **3,871,967**

[45] **Mar. 18, 1975**

[54] **SYNTHETIC NUTRIENT MEDIUM FOR MICROORGANISMS AND METHOD OF USE**

[75] Inventors: **Mohamed Abdou; Karl Buchta,** both of Ingelheim am Rhein, Germany

[73] Assignee: **Boehringer Ingelheim G.m.b.H.,** Ingelheim am Rhein, Germany

[22] Filed: **Jan. 11, 1974**

[21] Appl. No.: **432,546**

[52] **U.S. Cl.** ..... **195/100,** 195/103.5 R

[51] **Int. Cl.** **C12b 3/02,** C12b 3/12

[58] **Field of Search**..... 195/100, 101, 102, 103.5 R

[56] **References Cited**

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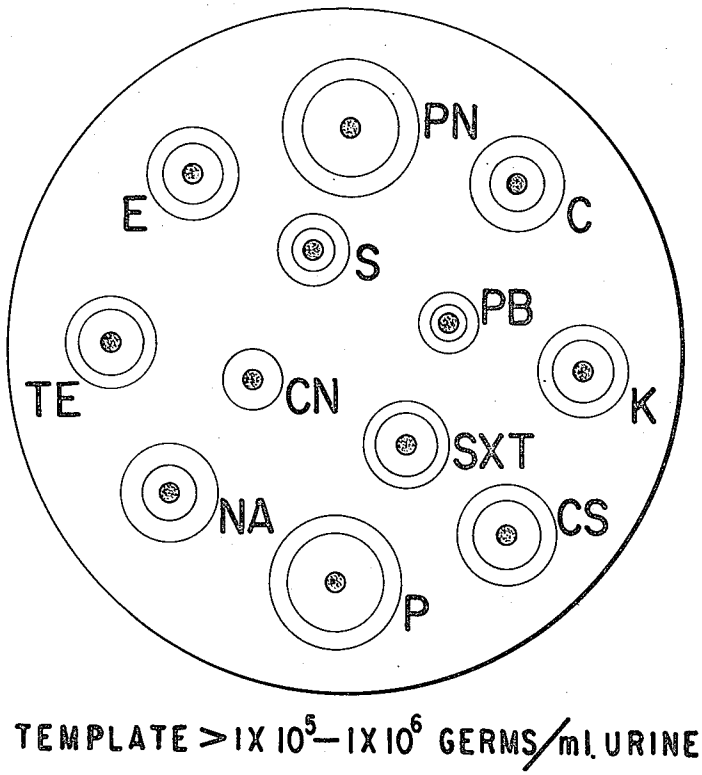
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Primary Examiner—Alvin E. Tanenholtz
Assistant Examiner—C. A. Fan
Attorney, Agent, or Firm—Hammond & Littell

[57] **ABSTRACT**

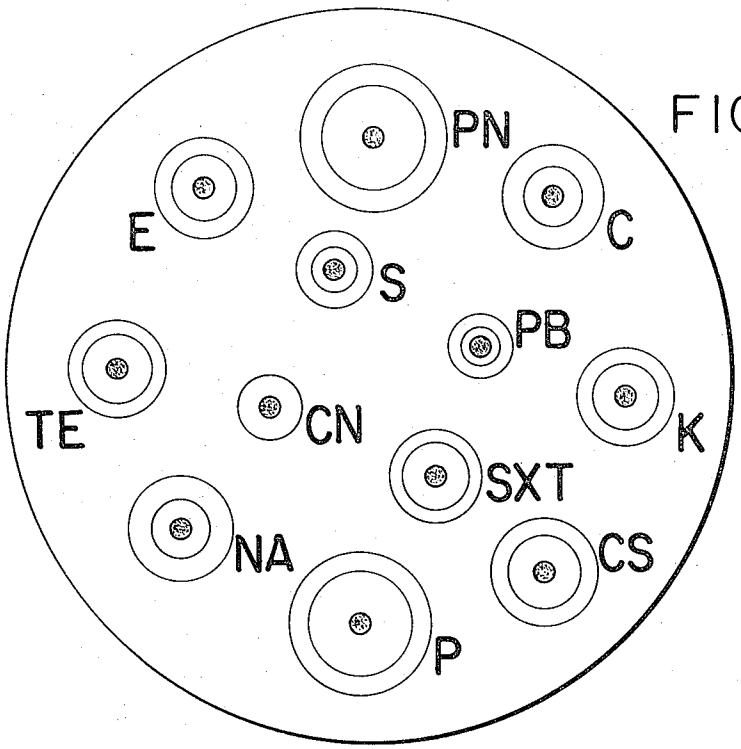
A nutrient medium composition for pathogenic microorganisms, especially for those which cause bacterial infections of the urinary tract, which is characterized in that it consists essentially of definitive chemical compounds, and such conventional nutrient medium components as peptones, meat extracts and yeast extracts are replaced by suitable amounts of aminoacids; the composition is useful for rapidly and simply testing the efficacy of chemotherapeutics against such pathogenic microorganisms with far greater accuracy than heretofore possible.

3 Claims, 2 Drawing Figures



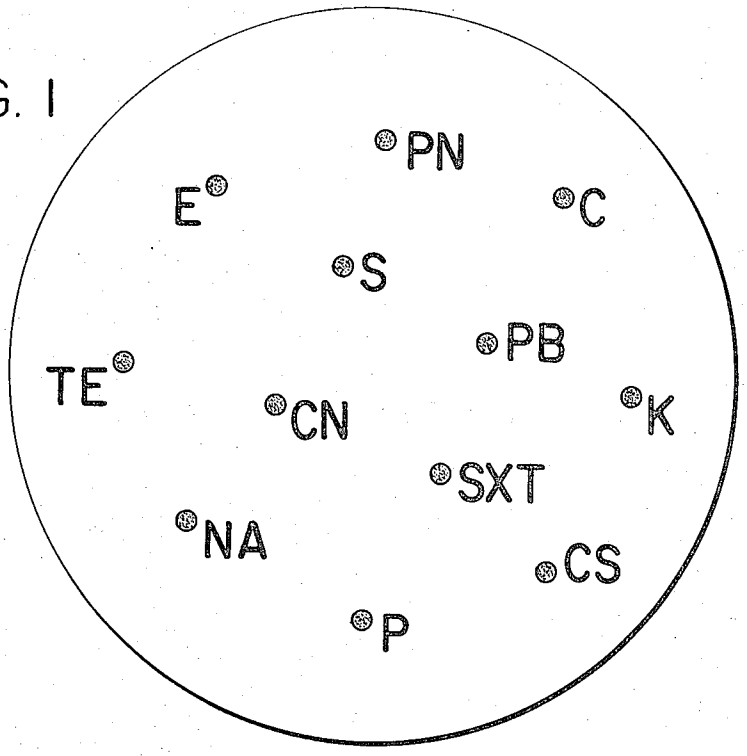
TEMPLATE > $1 \times 10^5 - 1 \times 10^6$ GERMS/ml. URINE

FIG. 2



TEMPLATE > $1 \times 10^5 - 1 \times 10^6$ GERMS/ml. URINE

FIG. 1



SYNTHETIC NUTRIENT MEDIUM FOR MICROORGANISMS AND METHOD OF USE

This invention relates to a synthetic nutrient medium composition for microorganisms, especially for the germs of bacterial urinary tract infections, and a method of using the same. By "synthetic" we mean that the nutrient medium is mainly composed of definitive chemical compounds; in particular, otherwise generally used mixtures of substances, such as peptones, meat extract, yeast extract, are replaced by suitable quantities of aminoacids.

The nutrient medium composition according to this invention is primarily useful for testing the susceptibility of the germs of bacterial urinary tract infections to chemotherapeutics and thus determine an effective chemotherapeutic treatment. Moreover, an approximate determination of the number of germs in test samples is made possible.

The easily reproducible properties of the nutrient medium composition allow for a standardization of the test; thus, it becomes possible to make more exact statements in an easy and simple manner regarding the applicability of a certain therapeutic than has been possible up to now.

The nutrient medium composition according to this invention may be composed as follows:

Solution I:

Components	gm/ltr. *)
L-arginine	0.523
L-asparaginic acid	1.065
L-cysteine	0.485
L-glutaminic acid	2.207
glycocoll	0.15
L-histidine	0.31
L-leucine	0.787
L-isoleucine	0.525
L-lysine	0.585
L-methionine	0.298
L-phenylalanine	0.33
L-proline	0.345
L-threonine	0.357
L-tryptophane	0.102
L-tyrosine	0.362
L-valine	1.17
NaCl	2.92
KCl	0.298
sodium acetate, free of H_2O	0.984
sodium pyruvate	1.1
sodium glycerophosphate . 5 H_2O	5.2
$MgSO_4$. 7 H_2O	0.049
adenine	0.009
guanine	0.009
uracil	0.011
agar, free of inhibitors and antagonists	10.0
aqua dist.	950.0 ml

Solution II:

Components	gm/ltr. *)
D-glucose	1.98
inosite	0.011
choline-Cl	0.014
nicotinic acid amide	0.011
thiamine-HCl	0.01
riboflavine	0.001
pyridoxin-HCl	0.006
pantothenic acid calcium salt	0.01
folic acid	0.009
biotin	0.0002
Na-glycerophosphate . 5 H_2O	1.04
p-nitrophenyl-propanetriol	0.005
pimaricin	0.1049
aqua dist.	50.0 ml

Solution II:-Continued

Components	gm/ltr. *)
Solution III:	
aqueous 10% sodium hydroxide solution	

*) The quoted quantities refer to the total quantity of distilled water used for preparing solutions I and II, that is, in the present example 950 ml plus 50 ml.

Preparation

1. The ingredients under Solution I are dissolved in 950 ml of distilled water, and the solution is sterilized in an autoclave at 121°C for 20 minutes.

2. The glucose, vitamins and growth promoters under Solution II are dissolved in 50 ml of distilled water and degerminated by sterile filtering over membrane filter, pore size 0.22 μm.

3. The 10% sodium hydroxide solution is sterilized in an autoclave for 20 minutes at 121°C.

4. Solutions I and II are each heated to 55°C and then admixed; with the aid of Solution III the pH-value of the mixture is adjusted to 7.2 at 47°C.

It is possible to choose different quantities of the components for Solutions I and II; the indicated amounts of the components may, for example, be reduced by one-half or doubled. However, in general, it is not advantageous to omit a component, for instance an aminoacid, completely or to materially alter the pH-value of the mixture. The nutrient media according to the invention can only be applied for a standardized test if the deviations do not materially affect the growth of the microorganisms and thus the size of the inhibition zones formed in the test described below.

The following advantages of the nutrient medium according to the invention should be particularly emphasized.

1. The susceptibility of the total germ population and the estimate of the total germ count can be determined simultaneously on one dish within 16 to 24 hours.

2. The species of the genus Proteus do not swarm.

3. Sulfonamides are not antagonized, cycloserines are not blocked, and tetracyclines are not inactivated.

4. Yeasts are excluded, and thus an incorrect reading of the antibiogram is prevented.

The nutrient medium according to this invention can be filled into sterilized Petri-dishes or test tubes while warm, and can be used in the conventional way.

However, its advantages are particularly revealed in the illustrative mode of application described below, the purpose of which is to find effective chemotherapeutics for the treatment of urinary tract infections; by analogous application of this illustrative mode, it is also possible to find effective therapeutics for the treatment of other bacterial infections.

First, 30 ml of the warm nutrient medium are evenly distributed in a sterilized Petri-dish of 140 mm diameter. After it has solidified, a seed layer is poured on this base layer (seed layer = 15 ml of nutrient medium plus 0.1 ml of urine containing pathogens which cause urinary tract infections), and the seed layer is allowed to solidify. Then, 12 round paper discs of about 4 mm diameter are put on the seed layer, each paper disc being charged with a certain amount of a different antimicrobially active chemotherapeutic; each active substance has a fixed place on the seed layer. The antimicrobially active substances are those of which a good action against the conventional pathogens which cause

infections of the urinary tract is expected. The dosage of chemotherapeutic with which the discs are charged is determined in accordance with therapeutic-pharmacological considerations.

cle of the template, gentamycin may be used as an effective therapeutic, otherwise not.

In the following table the values are indicated, on the basis of which the templates can be prepared.

Antimicrobial agent	Code designation	Disc charge	Inhibition zone diameter [mm.] depending on germ density								
			$>10^4$–10^5 germs/ml.			$>10^5$–10^6 germs/ml.			$>10^6$–10^7 germs/ml.		
			Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ampicillin	PN	10 μg	>30	>21–30	≦21	>28	>20–28	≦20	>26	>19–26	≦19
Chloramphenicol	C	30 μg	>21	>13–21	≦13	>20	>12–20	≦12	>19	>11–19	≦11
Kanamycin	K	30 μg	>21	>13–21	≦13	>20	>12–20	≦12	>19	>11–19	≦11
Cycloserin	CS	100 μg	>22	>16–22	≦16	>21	>15–21	≦15	>20	>14–20	≦14
Penicillin G	P	10 units	>30	>21–30	≦21	>28	>20–28	≦20	>26	>19–26	≦19
Nalidixic acid	NA	30 μg	>20	>13–20	≦13	>19	>12–19	≦12	>18	>11–18	≦11
Tetracycline	TE	30 μg	>20	>15–20	≦15	>19	>14–19	≦14	>18	>13–18	≦13
Erythromycine	E	15 μg	>19	>14–19	≦14	>18	>13–18	≦13	>17	>12–17	≦12
Streptomycine	S	10 μg	>16	>12–16	≦12	>15	>11–15	≦11	>14	>10–14	≦10
Polymyxin B	PB	300 units	>15	>10–15	≦10	>14	>9–14	≦9	>13	>9–13	≦9
Sulfamethoxazole/Trimethoprim	SXT	25 μg	>19	>14–19	≦14	>18	>13–18	≦13	>17	>12–17	≦12
Gentamycin	CN	10 μg	>15			>14			13		

FIG. 1 of the attached drawings is a top view of a Petri-dish containing the nutrient medium, seed layer and paper discs described above; the individual discs charged with the various antimicrobial substances are identified with abbreviations for the antimicrobial agents, were

PN = Ampicillin
C = Chloroamphenicol
K = Kanamycin
CS = Cycloserin
P = Penicillin G
NA = Nalidixic acid
TE = Tetracycline
E = Erythromycin
S = Streptomycin
PB = Polymyxin B
SXT= Sulfamethoxyzole/Trimethoprim
CN = Gentamycin

After an incubation period of 16 to 24 hours at 36 ± 1°C, the susceptibility category of the microorganisms indicated by the size of the inhibition zone, taking into account the germ density in the examimed sample, is determined with the aid of a standardized template.

The germ density (germs/ml) may be determined optically in known fashion, by comparing the density of the germ colony on the nutrient medium between the inhibition zones with the density of colonies in samples obtained with a fixed number of organisms. For the process according to the invention, a division into 3 ranges of germ density suffices ($>10^4$ – 10^5 organisms/ml; $>10^5$ 14 10^6 organisms/ml; $>10^6$ – 10^7 organisms/ml).

For each of the three ranges a standardized template is used for evaluation: the production thereof is explained further below. A template for the range above 10^5 – 10^6 organisms per ml is shown in FIG. 2 of the drawings. The black circles (representing the test discs) are surrounded by 2 concentric circles (except gentamycin). If the inhibition zone diameter is smaller than the inner circle, the organisms in the sample are resistant to the antimicrobially active substance. If the inhibition zone diameter lies between the diameter of the inner and the outer circle, a moderate effect of the antimicrobially active substance is indicated; however, the normal dose is then generally not sufficient for effective therapy. If the inhibition zone diameter is greater than the diameter of the outer circle of the template the antimicrobial substance is highly effective. in the case of gentamycin the middle range is not applicable; if the inhibition zone is greater than the single circle of the template, gentamycin may be used as an effective therapeutic, otherwise not.

The inhibition zone diameters given in the table are determined in the manner described below, using commercial test discs charged with standardized quantities of certain selected antimicrobial agents.

First, the minimal inhibitory concentration of the particular antimicrobial agent and the size of the inhibition zone diameter produced by the test disc at a certain germ density are determined for as many strains of the types of organisms under consideration as possible. The trial with the test discs is carried out in the same way as later in the standardized test (described above). There is a correlation between the minimal inhibitory concentration and the inhibition zone diameter, which can be represented by a regression curve; the greater the minimal inhibitary concentration, the smaller the inhibition zone diameter (at a given antimicrobial agent charge of the test discs). The concentration of antimicrobial agent achievable in the blood or infected tissues, however, is not unlimited because it depends upon the safe dosage of the therapeutic agent. Therefore, only those organisms can be combatted by a given therapeutic agent, the minimal inhibitory concentration of which is lower than the highest therapeutic concentration attainable in the blood or infected tissues. Every minimal inhibitory concentration corresponds to an inhibition zone of a certain diameter.

If a larger inhibition zone is formed in the test, this indicates a lower minimal inhibitory concentration of the microorganism, which may then be effectively combatted with the particular therapeutic agent. However, if the inhibition zone is smaller, the germ is less sensitive toward the therapeutic agent (intermediate) or resistant. In this case the antimicrobial agent is unsuitable or less suitable for therapeutic treatment of the infection.

A suitable embodiment of the process according to the invention consists of using as a carrier for the nutrient medium a sufficiently absorbant cardboard disc, 1 to 2 mm thick, covered with a membrane filter. The cardboard disc may be covered with the membrane filter after impregnation with the nutrient medium and drying. It is of advantage to use the type of cardboard and membrane filter assembly which is usually used for germ-free filtration. In order to provide greater contrast of the inhibition zone against the background, the

membrane filters may be dyed with a contrasting color. The impregnated cardboard discs are dried carefully, for example in vacuo or by freeze-drying. The nutrient medium may be used for this purpose without the addition of agar. In order to impregnate a cardboard disc of 140 mm diameter and 1.5 mm thickness, about 15 ml of nutrient medium are required. The nutrient cardboard discs are packed sterile and then remain stable for a long time. For use, they are simply put into sterile Petri-dishes of a suitable size and moistened. Moistening and applying of the seed layer are combined in a suitable way, by mixing the urine sample to be examined with the moistening liquid, and adding the mixture to the nutrient cardboard. For one nutrient cardboard disc of the size indicated above, 15 ml of moistening liquid are required (for example, sterile water, sterile physiological sodium chloride solution or diluted nutrient medium). Standardization and evaluation are effected as described above. Instead of using a standardized template, nutrient cardboard discs with differently dyed indices printed thereon may be employed.

While the present invention has been illustrated with the aid of certain specific embodiments thereof, it will be readily apparent to others skilled in the art that the invention is not limited to these particular embodiments, and that various changes and modifications may be made without departing from the spirit of the invention or the scope of the appended claims.

We claim:

1. A nutrient medium composition for microorganisms, in particular for germs which cause urinary tract infections, consisting essentially of water and about 0.25 to 1 gm of L-arginine, 0.5 – 2 gm of L-asparaginic acid, 0.25 – 1 gm of L-cysteine, 1 – 4 gm of L-glutaminic acid, 0.1 – 0.4 gm of glycocoll, 0.15 – 0.6 gm of L-histidine, 0.4 – 1.6 gm of L-leucine, 0.25 – 1 gm of L-isoleucine, 0.2 – 1.3 gm of L-lysine, 0.15 – 0.6 gm of L-methionine, 0.15 – 0.6 gm of L-phenylalanine, 0.2 – 0.8 gm of L-proline, 0.2 – 0.8 gm of L-threonine, 0.05 – 0.2 gm of L-tryptophane, 0.2 – 0.8 gm of L-tyrosine, 0.5 – 2 gm of L-valine, 1.5 – 6 gm of sodiumchloride, 0.15 – 0.6 gm of potassium chloride, 0.5 – 2 gm of anhydrous sodium acetate, 0.5 – 2 gm of sodium pyruvate, 3 – 12 gm of sodium glycerophosphate pentahydrate, 0.025 – 0.1 gm of magnesiumsulfate heptahydrate, 0.005 – 0.02 gm of adenine, 0.005 – 0.02 gm of guanine, 0.005 – 0.02 gm of uracil, 1 – 4 gm of D-glucose, 0.005 – 0.02 gm of inosite, 0.005 – 0.02 gm of choline chloride, 0.005 – 0.02 gm of nicotinic acid amide, 0.005 – 0.02 gm of thiamine hydrochloride, 0.0005 – 0.002 gm or riboflavin, 0.003 – 0.012 gm of pyridoxin hydrochloride, 0.005 – 0.02 gm of calcium pantothenate, 0.005 – 0.02 gm of folic acid, 0.0001 – 0.0004 gm of biotin, 0.0025 – 0.01 gm of p-nitrophenylpropanetriol, 0.05 – 0.2 gm of pimaricin per liter of water, as well as a quantity of dilute aqueous sodium hydroxide dilution necessary for adjusting the pH of the composition to 7.2, and optionally a quantity of agar (free from germ growth inhibiting or antimicrobial agent antagonizing substances) necessary for solidification of the nutrient medium.

2. A nutrient medium composition of claim 1, free from said agar component, said composition being applied to carrier substrate which consists of cardboard covered with a membrane filter.

3. A nutrient medium composition of claim 2 which has been converted into a stable, storable form by drying and sterile packaging.

* * * * *

UNITED STATES PATENT OFFICE

CERTIFICATE OF CORRECTION

Patent No. 3,871,967 Dated March 18, 1975

Inventor(s) MOHAMED ABDOU and KARL BUCHTA

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Title Page - Insert

--[30] Foreign Application Priority Data

Jan. 11, 1973 Germany P 23 01 211.5--

Signed and sealed this 20th day of May 1975.

(SEAL)
Attest:

RUTH C. MASON
Attesting Officer

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