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Original Article

A suitable blood agar containing human blood especially for the use in laboratories of developing countries

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Abstract

Introduction: In developed countries, blood agar containing defibrinated sheep or horse blood is a standard tool for the isolation of bacteria from clinical samples. Several issues prevent blood agar containing animal blood from being used in many developing countries. However, the use of easily available human blood for blood agar is discouraged because of the common tenet that human blood in nutrient media results in poor bacterial isolation rates and hardly visible hemolysis or no hemolysis at all. We have developed a reconfigured and easily applicable composition for blood agar containing human blood and tested its usability with respect to hemolysis visibility and its characteristics in antibiograms with *Streptococcus* spp.

Methodology: Hemolysis tests were conducted with clinical strains of *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus mitis*. In a second test series, clinical strains of *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, and *Streptococcus mitis* were tested with Mueller-Hinton agars containing defibrinated wether blood as well as with Mueller-Hinton agars containing citrated human blood to compare the results of antibiotic susceptibility testing.

Results: The reconfigured blood agars containing 2.5% citrated human blood showed almost identical reactions to the standard blood agars used in the developed world.

Conclusions: For the first time, blood agars containing 2.5% citrated human blood were shown to be an acceptable alternative for the isolation of the above-mentioned bacteria as well as for use in antibiotic susceptibility testing.

Key words: blood agar; human blood; bacteria isolation; hemolysis; antibiograms.

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Introduction

The gold standard for diagnosing hemolyzing bacteria such as beta-hemolytic *Staphylococcus aureus* and beta-hemolytic *Streptococcus* spp. is cultivating these organisms on solid culture media containing a source of blood that can be hemolyzed. To prepare this blood agar, defibrinated sheep, goat, pig or horse blood in a concentration of 5% [1-3] is recommended. However, blood agar containing animal blood is not a feasible option in many developing countries due to technical and personnel issues as well as the high cost and adverse climate conditions for raising these animals [1,4]. Therefore, common practice in many developing countries is to prepare blood agar using human blood, especially taken from expired blood transfusion bags or from volunteers, often the laboratory technicians themselves [3-4]. This occurs also in Sierra Leone and Liberia, where we observed human blood being used as the main source for preparing blood agar.

However, the use of human blood for blood agar is not recommended [3-6]. The common tenet is that human blood in nutrient media results in poor bacterial isolation rates and in hardly visible hemolysis or no hemolysis at all [3,5]. Citric acid, an anticoagulant and a substitute for difficult-to-achieve defibrination, is often mentioned as one possible cause for this phenomenon [3]. Blood bags often contain citric acid, which is also a well-known bacteriostatic agent.

Because of the above-mentioned problems concerning animal blood and the easy, cheap, and convenient availability of human blood (*e.g.*, from expired blood bags), we wanted to develop a reconfigured and easily applicable composition for a blood agar containing human blood. The starting point of our work was the fact that human blood has a much higher average hemoglobin (Hb) value (around 15 g/dL [7]) than does sheep blood (average Hb value of around 8 g/dL [8]). Additionally, defibrinating sheep blood probably causes a further decrease of the Hb value.

With this in mind, it is conceivable that hemolysis on blood agar containing human blood is present but not visible due to an oversupply of hemoglobin. We therefore worked on a new composition for blood agar with a significantly lower concentration of human blood. This nutrient medium was then tested for its performance characteristics when cultivating beta-hemolytic *Staphylococcus aureus* and beta-hemolytic *Streptococcus* spp. We also tested its suitability for antibiotic susceptibility testing for several strains of alpha- and beta-hemolytic *Streptococcus* spp.; however, our focus was the hemolysis visibility of the new reconfigured blood agar when cultivating beta-hemolytic *Staphylococcus aureus* and *Streptococcus* spp. This decision was based on the fact that no blood agar is needed for susceptibility tests for *Staphylococcus aureus* and that an alternative medium, standard nutrient agar 1, is available for susceptibility tests for *Streptococcus* spp. [9].

Methodology

The study was conducted at the laboratory of the non-governmental organization Globolab e.V., in Assling, Germany, and at the laboratory of the hospital Saint John of God, Lunsar-Mabesseneh, Sierra Leone. The samples were obtained from patients admitted to a cooperating hospital in Germany and from patients admitted to the hospital Saint John of God, Lunsar-Mabesseneh, Sierra Leone. All samples were taken after consent had been obtained and were anonymized prior to testing. All blood donations were obtained with consent. The first streaking and susceptibility testing was performed at the Globolab e.V. laboratory, and comparative testing took place at the hospital Saint John of God, Lunsar-Mabesseneh, Sierra Leone.

Selection, collection, and isolation of bacterial strains for hemolysis testing at the Globolab e.V. laboratory

For testing the visibility of hemolysis on the reconfigured blood agar containing a lower concentration of human blood, three different strains of hemolytic bacteria and one non-hemolytic (gamma-hemolytic) strain as negative control were used: beta-hemolytic *Staphylococcus aureus*, beta-hemolytic *Streptococcus dysgalactiae*, alpha-hemolytic *Streptococcus mitis*, and gamma-hemolytic *Enterococcus faecalis*.

All strains were isolated from clinical samples on ready-to-use blood agar plates containing Columbia agar enriched with 5% wether blood. These ready-to-use blood agar plates were obtained from bioMérieux (Nürtingen, Germany). Species classification was performed with the API identification system Rapid ID 32 (bioMérieux, Nürtingen, Germany).

Laboratory methods for hemolysis testing at the Globolab e.V. laboratory

To compare the visibility of hemolysis on the different media, one colony of each bacterial family (each colony taken from the third sector of the three-sector streak on the cultivation media) was streaked in duplicate on three types of agar with an inoculation loop:

(1) Columbia agar enriched with 5% defibrinated sheep blood (dSBA5.0) as a reference standard; (2) Columbia agar enriched with 5% citrated human blood (cHuBA5.0); and (3) Columbia agar enriched with 2.5% citrated human blood (cHuBA2.5), the reconfigured nutrient medium.

Blood collection, medium preparation, and hemolysis testing at the Globolab e.V. laboratory

To mimic the circumstances in developing countries, citrated human blood was taken instead of defibrinating the human blood. Defibrination is impossible in many developing countries, while conveniently available blood from expired blood bags is citrated. The defibrinated sheep blood was obtained from Oxoid (Wesel, Germany). The citrated human blood was taken from donors using 3 mL S-Monovettes from Sarstedt AG & Co. (Nümbrecht, Germany) and used immediately. These blood collection tubes contained 0.30 mL trisodium citrate solution with a concentration of 0.106 mol/L.

All hemolysis tests were conducted twice. One test series was done with blood from a female donor with blood group 0 Rh D-positive and one test series was done with blood from a male donor with blood group A Rh D-negative.

Columbia agar was obtained from Carl Roth GmbH + Co. KG (Karlsruhe, Germany), in the form of a granular base. It contained casein peptone (pancreatic digest), 10.0 g/L; beef peptone (peptic digest), 5.0 g/L; heart peptone (pancreatic digest), 3.0 g/L; yeast extract, 5.0 g/L; corn starch, 1.0 g/L; sodium chloride, 5.0 g/L; and agar, 13.5 g/L.

The nutrient matrix was prepared based on the manufacturer's instructions. After sterilization and cooling down to 47°C, the defibrinated sheep blood and the citrated human blood were added separately in the above-mentioned concentrations. Sterile glass petri dishes were filled with the different nutrient media and dried.

After solidifying and drying the agar plates, the different nutrient media were streaked with three sector streaks as mentioned above. Subsequently, the plates were incubated in a Memmert UE 200 incubator for 20 hours at 35°C under ambient air. Ambient air was chosen instead of a CO_2-containing atmosphere because this is the common way in developing countries. After incubation, the appearance of the colonies and their hemolysis were evaluated visually, the appearance of the colonies under impinging light, and hemolysis under transmitted light. The colony diameters and the area of the hemolysis were measured electronically using photographs.

Selection, collection, and isolation of bacterial strains for antibiotic susceptibility testing at the Globolab e.V. laboratory

For testing the applicability of the reconfigured blood agar containing 2.5% citrated human blood for the Kirby-Bauer disk diffusion test (antibiogram), six different strains of alpha- and beta-hemolytic *Streptococcus* spp. were used: beta-hemolytic *Streptococcus anginosus*, beta-hemolytic *Streptococcus dysgalactiae*, beta-hemolytic *Streptococcus agalactiae*, alpha-hemolytic *Streptococcus mitis*, beta-hemolytic *Streptococcus dysgalactiae*, and beta-hemolytic *Streptococcus dysgalactiae*.

These strains were isolated from clinical samples on ready-to-use blood agar plates and classified with the API identification system as explained above. Susceptibility testing for *S. aureus* was not performed, as blood is not required as a medium supplement for this type of pathogen.

Laboratory methods for comparing the media during susceptibility testing at the Globolab e.V. laboratory

To evaluate whether the reconfigured medium is a suitable substitute for the standard medium, identical antibiograms on the two different agars were compared. Each of the above-mentioned strains was inoculated onto two different Mueller-Hinton blood agar plates (Carl Roth GmbH + Co. KG, Karlsruhe, Germany): (1) Mueller-Hinton agar enriched with 5% defibrinated sheep blood (dSBMHA5.0), the reference standard; and (2) Mueller-Hinton agar enriched with 2.5% citrated human blood (cHuBMHA2.5), the reconfigured nutrient medium.

The following paper disks impregnated with different antibiotics, obtained from Oxoid Deutschland GmbH (Wesel, Germany), were applied each on dSBMHA5.0 and on cHuBMHA2.5: amoxicillin/clavulanic acid (AMC), 20/10 µg; cefuroxime (CXM), 30 µg; erythromycin (E), 15 µg; gentamicin (CN), 10 µg; imipenem (IMP), 10 µg; levofloxacin (LEV), 5 µg; oxacillin (OX), 1 µg; vancomycin (VA), 30 µg.

To compare the two nutrient media as antibiotic susceptibility test agars, the method described in a previous work [9] were used. In brief, to analyze the growth performances and inhibition characteristics of two different nutrient media, a series of identical antibiograms on the two media were prepared. The average diameter of the zones of inhibition (ZoI) of one medium was compared with the average ZoI diameter of the second medium. For the comparison, signed values of the ZoI differences were used, because a ZoI difference between two culture media is an indication of either inhibition (negative difference) or increase (positive difference) of growth. For this reason, the algebraic signs of the ZoI differences cannot be disregarded.

Medium preparation and susceptibility testing at the Globolab e.V. laboratory

Mueller-Hinton agar was obtained from Carl Roth GmbH + Co. KG (Karlsruhe, Germany), in the form of a granular base. It contained beef infusion, 2.0 g/L; corn starch, 1.5 g/L; acid casein peptone, 17.5 g/L; and agar, 17.0 g/L.

The nutrient matrix was prepared according to the manufacturer's instructions. After sterilization and cooling down to 47°C, the defibrinated sheep blood and the citrated human blood were added separately in the above-mentioned concentrations. For the preparation of the blood supplemented with Mueller-Hinton agar, the same blood was used as for the blood supplemented with Columbia agar. Sterile glass petri dishes were filled with the nutrient media and dried. The different strains were inoculated onto the different blood Mueller-Hinton plates, based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST)'s instructions [2]. Subsequently, all plates were incubated for 22 hours at 35°C under ambient air. After incubation, the zone diameters were read by two scientists independently.

Results

The results of the hemolysis tests with blood agars containing blood from the above-mentioned two donors with different blood types were identical. Table 1 shows colony appearances and zones of hemolysis for tested strains on three different blood agar plates.

Figure 1. Growth of *Staphylococcus aureus* on the three different blood agars.

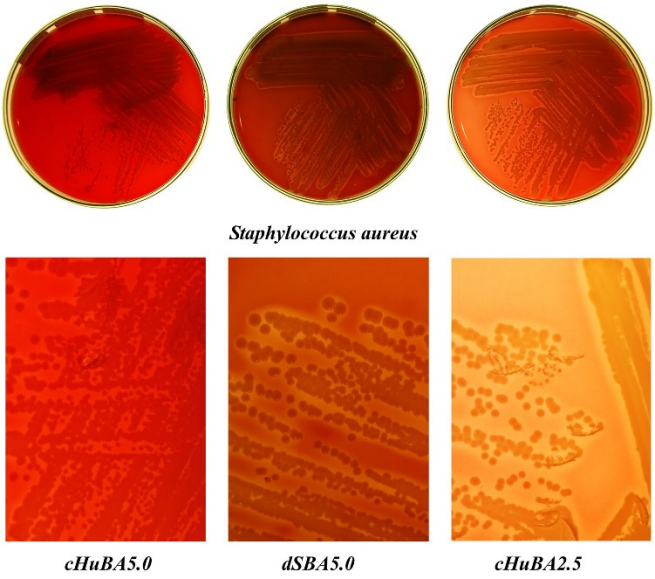
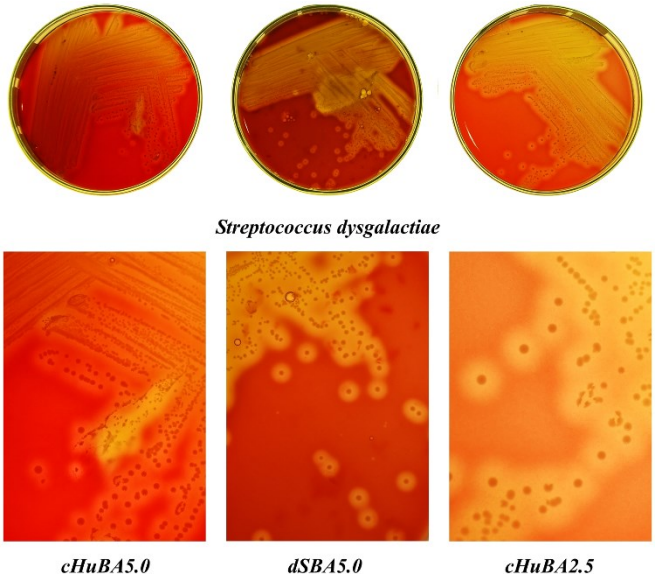


Figure 2. Growth of *Streptococcus dysgalactiae* on the three different blood agars.



Staphylococcus aureus

The numbers of colonies of *S. aureus* strains were similar on dSBA5.0 and cHuBA2.5. On cHuBA5.0, the colony number for *S. aureus* seemed a little lower. However, the difference was so small that this may have been the result of slightly different streaking. Colony appearances and colony sizes were identical on dSBA5.0, cHuBA5.0, and cHuBA2.5. The typical slightly golden color of *S. aureus* colonies was also visible on cHuBA2.5. According to the common tenet, beta-hemolysis of *S. aureus* was almost absent on cHuBA5.0, but it was obvious on cHuBA2.5 as well as on the reference agar dSBA5.0 (Figure 1).

Streptococcus dysgalactiae

The numbers of colonies of *S. dysgalactiae* strains were similar on dSBA5.0, cHuBA5.0, and cHuBA2.5. The colony appearances were nearly identical on all three agars; only on cHuBA5.0 it seemed a little less bright. Colony sizes were also nearly identical. On cHuBA5.0, they were a little larger, but the difference was so small that this could have been the result of normal biological variance. Beta-hemolysis of *S. dysgalactiae* was only faint on cHuBA5.0, but it was obvious on cHuBA2.5 as well as on the reference agar dSBA5.0 (Figure 2).

Table 1. Comparison of colony appearances and zones of hemolysis for tested strains on three different blood agars.

Strain and characteristics	Colony appearances and zones of hemolysis on different blood agar plates		
	cHuBA5.0	**dSBA5.0**	**cHuBA2.5**
Staphylococcus aureus			
Colony appearance	Shiny, opaque, golden	Shiny, opaque, golden	Shiny, opaque, golden
Colony size (mm) (*)	1.2 - 1.7	1.2 - 1.7	1.2 - 1.7
Beta-hemolysis	Almost absent	Obvious	Obvious
Streptococcus dysgalactiae			
Colony appearance	Dry, grey	Dry, grey-white	Dry, grey-white
Colony size (mm) (*)	1.0 - 1.2	1.0	1.0
Beta-hemolysis	Faint	Obvious	Obvious
Streptococcus mitis			
Colony appearance	Dry, grey-brown	Dry, grey	Dry, grey
Colony size (mm) (*)	1.0	1.0	0.8 - 1.0
Alpha-hemolysis	Almost absent	Obvious	Obvious
Enterococcus faecalis			
Colony appearance	Slightly transparent, grey	Slightly transparent, grey	Slightly transparent, grey
Colony size (mm) (*)	1.0 - 1.3	1.0 - 1.2	1.0 - 1.2
Hemolysis	Absent	Absent	Absent

(*) Colony size in the third sector of the three loop streak, electronically measured on the basis of photographs.

Streptococcus mitis

The numbers of colonies of *S. mitis* strains were similar on dSBA5.0, cHuBA5.0, and cHuBA2.5. The colony appearances were nearly identical on all three agars; only on cHuBA5.0 it seemed a little more brown. Colony sizes were also nearly identical. On cHuBA2.5 they were a little smaller, but the difference was so small that this could have been the result of normal biological variance. Alpha-hemolysis of *S. mitis* was almost absent on cHuBA5.0 according to the common tenet, but it was obvious on cHuBA2.5 as well as on the reference agar dSBA5.0 (Figure 3).

Enterococcus faecalis

Gamma-hemolytic *E. faecalis* was cultured as a negative control to confirm the absence of hemolysis on all agars. The numbers of colonies of *E. faecalis* strains were similar on dSBA5.0, cHuBA5.0, and cHuBA2.5. Colony sizes and appearances were also identical on all three agars. Hemolysis of *E. faecalis* was absent on dSBA5.0, cHuBA5.0, and cHuBA2.5.

Antibiotic susceptibility testing

Using existing tables for interpreting the ZoI on dSBMHA5.0 to interpret the ZoI on cHuBMHA2.5 requires *Streptococcus* spp. to have the same or at least very similar growth and inhibition characteristics on both nutrient media. If it were possible to demonstrate almost identical growth and inhibition characteristics on dSBMHA5.0 and on cHuBMHA2.5, existing tables for interpreting the diameters on dSBMHA5.0 could be used to interpret the ZoI diameters on cHuBMHA2.5. Table 2 shows the comparison of ZoI developed by *Streptococcus* spp. on dSBMHA5.0 and cHuBMHA2.5.

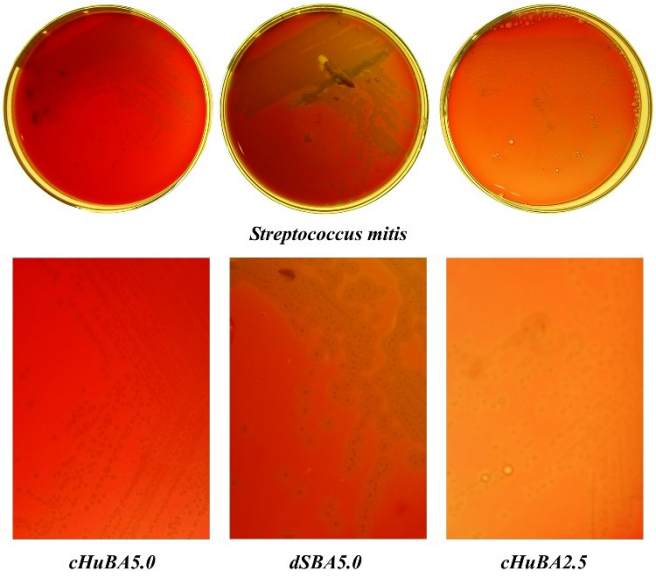
Statistical summary

The sum of all differences between dSBMHA5.0 and cHuBMHA2.5 was 19 mm. Thus, the average ZoI difference between the two media was 0.39 mm, calculated on the basis of signed values (∑ ZoI differences / no. of tests).

As shown in Table 2, ZoI diameters between 6 mm and 45 mm were found on the dSBMHA5.0 plates. The average ZoI diameter on the dSBMHA5.0 plates (∑ all ZoI on dSBMHA5.0/ no. of all ZoI on dSBMHA5.0 plates) was 23.1 mm. Therefore, an average ZoI difference of 0.39 mm was 1.7 % of the average ZoI diameter on dSBMHA5.0.

Finally, it should to be noted that there was no significant spike for any antibiotic agent. This means that there was no antibiotic agent causing an agent-specific idiosyncrasy on only one of the two nutrient media.

Figure 3. Growth of *Streptococcus mitis* on the three different blood agars.



Interpretation of the statistical summary

Firstly, differences in biological systems are to be expected. Secondly, EUCAST recommends rulers, calipers, or automatic zone readers for measuring ZoI diameters [2]. However, with the use of semi-opaque agar plates and frequently appearing blurred edges between bacterial growth and inhibition zones, measuring errors of a few millimeters are common [10]. Furthermore, with automatic zone readers, errors of up to 3 mm are accepted [11]. In summary, differences in ZoI diameter of up to 2 or 3 mm are acceptable. The average ZoI difference of 0.39 mm between dSBMHA5.0 and cHuBMHA2.5 suggests that *Streptococcus* spp. show very similar growth and inhibition behavior on the two different antibiogram media. Therefore, the existing tables for interpreting the ZoI of *Streptococcus* spp. on dSBMHA5.0 can also be consulted for interpretation of antibiograms performed on cHuBMHA2.5.

Comparative tests in Sierra Leone

To demonstrate the suitability of cHuBA2.5 when produced and used in developing countries, nine comparative tests were conducted in the laboratory of the hospital St. John of God, Mabesseneh-Lunsar, Sierra Leone. Hemolysis visibility of three different strains of beta-hemolytic *Staphylococcus aureus* was tested on three batches of cHuBA2.5, each containing human blood from a different indigenous donor.

Table 2. Comparison of ZoI in mm developed by *Streptococcus* spp. on dSBMHA5.0 and cHuBMHA2.5 agar.

Test No	Strain No.	Antibiotic	ZoI (dSBMHA5.0)	ZoI (cHuBMHA2.5)	Δ ZoI
1	1	AMC	30	30	0
2	1	CXM	36	36	0
3	1	E	30	30	0
4	1	CN	30	30	0
5	1	IMP	40	40	0
6	1	LEV	30	30	0
7	1	OX	14	14	0
8	1	VA	22	24	2
9	2	AMC	30	30	0
10	2	CXM	27	28	1
11	2	E	17	18	1
12	2	CN	12	16	4
13	2	IMP	30	30	0
14	2	LEV	18	19	1
15	2	OX	17	18	1
16	2	VA	17	18	1
17	3	AMC	30	30	0
18	3	CXM	45	39	-6
19	3	E	28	28	0
20	3	CN	12	14	2
21	3	IMP	36	37	1
22	3	LEV	19	19	0
23	3	OX	16	19	3
24	3	VA	19	19	0
25	4	AMC	23	23	0
26	4	CXM	22	24	2
27	4	E	6	6	0
28	4	CN	20	22	2
29	4	IMP	26	30	4
30	4	LEV	10	10	0
31	4	OX	6	6	0
32	4	VA	23	24	1
33	5	AMC	32	32	0
34	5	CXM	30	30	0
35	5	E	18	19	1
36	5	CN	18	18	0
37	5	IMP	34	36	2
38	5	LEV	20	20	0
39	5	OX	20	20	0
40	5	VA	19	20	1
41	6	AMC	28	28	0
42	6	CXM	30	30	0
43	6	E	24	24	0
44	6	CN	12	12	0
45	6	IMP	30	30	0
46	6	LEV	18	18	0
47	6	OX	16	10	-6
48	6	VA	21	22	1
				Σ Δ ZoI (signed values):	+ **19 mm**

Table 3. Comparison of colony appearances and zones of hemolysis of three different strains of beta-hemolytic *S. aureus* on cHuBA2.5 prepared with blood from three different Sierra Leonean blood donors and on standard dSBA5.0.

Strain and characteristics	Colony appearances and zones of hemolysis on blood agar prepared with blood from three different blood donors B1, B2 and B3 and on dSBA5.0			
	cHuBA2.5 – B1	cHuBA2.5 – B2	cHuBA2.5 – B3	dSBA5.0
***S. aureus* Strain 1**				
Colony appearance	Dry, opaque, white-grey	Dry, opaque, white-grey	Dry, opaque, white-grey	Dry, opaque, white-grey
Colony size (mm) (*)	1.2 - 1.6	1.2 - 1.5	1.0 - 1.5	1.0 - 1.7
Beta-hemolysis	Sufficient obvious	Sufficient obvious	Obvious	Obvious
***S. aureus* Strain 2**				
Colony appearance	Dry, opaque, golden	Dry, opaque, golden	Dry, opaque, golden	Dry, opaque, golden-white
Colony size (mm) (*)	1.2 - 1.9	1.5 - 2.0	1.5 - 2.0	1.2 - 1.8
Beta-hemolysis	Obvious	Obvious	Obvious	Obvious
***S. aureus* Strain 3**				
Colony appearance	Dry, opaque, white-grey	Dry, opaque, grey	Dry, opaque, grey	Dry, opaque, grey
Colony size (mm) (*)	1.0 - 1.9	1.0 - 2.0	1.0 - 1.8	1.2 - 2.0
Beta-hemolysis	Obvious	Obvious	Obvious	Obvious

(*) Colony size in the third sector of the three loop streak, measured with ruler, partially estimated.

As is well known, beta-hemolytic *S. aureus* normally develops a less pronounced hemolysis than beta-hemolytic *Streptococcus* spp., and therefore beta-hemolytic *S. aureus* is particularly suited to this purpose.

For the first test series, blood from a female donor with blood group A Rh D-positive and with an Hb value of 12.2 g/dL (cHuBA2.5 – B1) was used. For the second test series, blood from a female donor with blood group 0 Rh D-positive (Hb 13.8 g/dL; cHuBA2.5 – B2) was used, and for the third test series, blood from a male donor with blood group B Rh D-positive (Hb 15.5 g/dL; cHuBA2.5 – B3) was used. These cHuBA2.5 agars were prepared as described above.

Each of the three different cHuBA2.5 batches was prepared in duplicate for each strain. Each series of six dishes was then streaked with the relevant strain of beta-hemolytic *S. aureus*: strain 1 – swab, soft tissue surgery; strain 2 – infected wound; strain 3 – infected wound.

For comparison purposes, all three strains were also streaked on ready-to-use dSBA5.0 plates obtained from bioMérieux (Nurtingen, Germany). These dSBA5.0 plates contained Columbia agar + 5% wether blood.

Table 3 shows that the results obtained with cHuBA2.5 prepared in Sierra Leone are almost identical to the results shown in Table 1 and Figure 1. Colony appearances and zones of hemolysis of the various strains of *S. aureus* tested on three different cHuBA2.5 batches (cHuBA2.5 – B1, cHuBA2.5 – B2, and cHuBA2.5 – B3) containing blood from different indigenous blood donors are comparable to the appearances and hemolysis visibility on dSBA5.0. In particular, the typical, slightly golden color of strain 2 *S. aureus* was also visible on the Sierra Leone cHuBA2.5. The other two *S. aureus* strains, strain 1 and strain 3, showed no golden color, not even on dSBA5.0.

Further observation

Although trisodium citrate is the anticoagulant of choice in blood collection bags in developing countries, EDTA and heparin as anticoagulants for blood to be used in blood agar were tested. However, these anticoagulants were already discarded in the run-up of our study, because blood agar containing human blood anticoagulated with EDTA and heparin developed no visible (EDTA) or very faintly visible (heparin) hemolysis in both the 5.0% and the 2.5% concentrations. Also, freshly taken human blood without anticoagulant seemed to be inferior to citrated blood.

Discussion

The results presented here confirm that *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, and *Streptococcus mitis* effectively grow on blood agar containing human blood. Moreover, they developed clearly visible hemolysis on the reconfigured nutrient medium

Table 4. Suggested addition of citrated human blood to agars depending on the Hb value of the used blood.

Hb value of used blood	Blood added to agar
Hb: 9.5 g/dl	3.25%
Hb: 11.0 g/dl	3.00%
Hb: 12.5 g/dl	2.75%
Hb: 14.0 g/dl	2.50%
Hb: 15.5 g/dl	2.25%

cHuBA2.5. Also, colony size and colony appearance on cHuBA2.5 was very similar compared to the standard dSBA5.0; finally, there was no significant difference between the antibiograms on cHuBMHA2.5 and on dSBMHA5.0.

The above results show that in addition to the use of citrated blood, the hemoglobin concentration and not the blood concentration seems to be the key factor in the preparation of blood agar. We therefore suggest to make the amount of added blood dependent on the Hb value of the blood, cf. examples in Table 4.

The described findings have profound implications for developing countries, where an easily applicable blood agar can now be prepared with human blood, using citrated human blood freshly taken from blood donors or perhaps even blood from expired human blood bags. Each person donating blood for preparing blood agar must be screened for infectious diseases they same way conventional blood donors are screened. Moreover, it must be ensured that donors have not been treated with any antibiotics for at least one month prior to donating blood.

Previous studies have described blood agar containing human blood as inferior to agar with sheep or horse blood. It has been suggested that human blood may contain antibiotics, antibodies, or other anti-infective agents [3] and the lack of hemolysis on agar containing human blood may be due to the age of the red blood cells in the expired human blood or some other factor [3]. Based on the above results, an easily applicable blood agar containing readily available human blood is available for the first time – a nutrient medium that most developing countries can prepare without requiring a major investment.

Conclusions

As mentioned before, blood agar containing animal blood is not a feasible option in many developing countries. On the other hand, experts have so far considered human blood unsuitable for the preparation of blood agar. Our studies show for the first time that blood agars containing 2.5% citrated human blood are an acceptable alternative for the isolation of *Streptococcus* spp. and *Staphylococcus* spp. as well as for use in antibiotic susceptibility testing of *Streptococcus* spp. Thus, laboratories in developing countries are now able to prepare suitable blood agar. In the future this new reconfigured blood agar will enable laboratories in developing countries to approach western standards.

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