

Optimization and standardization of the culturomics technique for human microbiome exploration.

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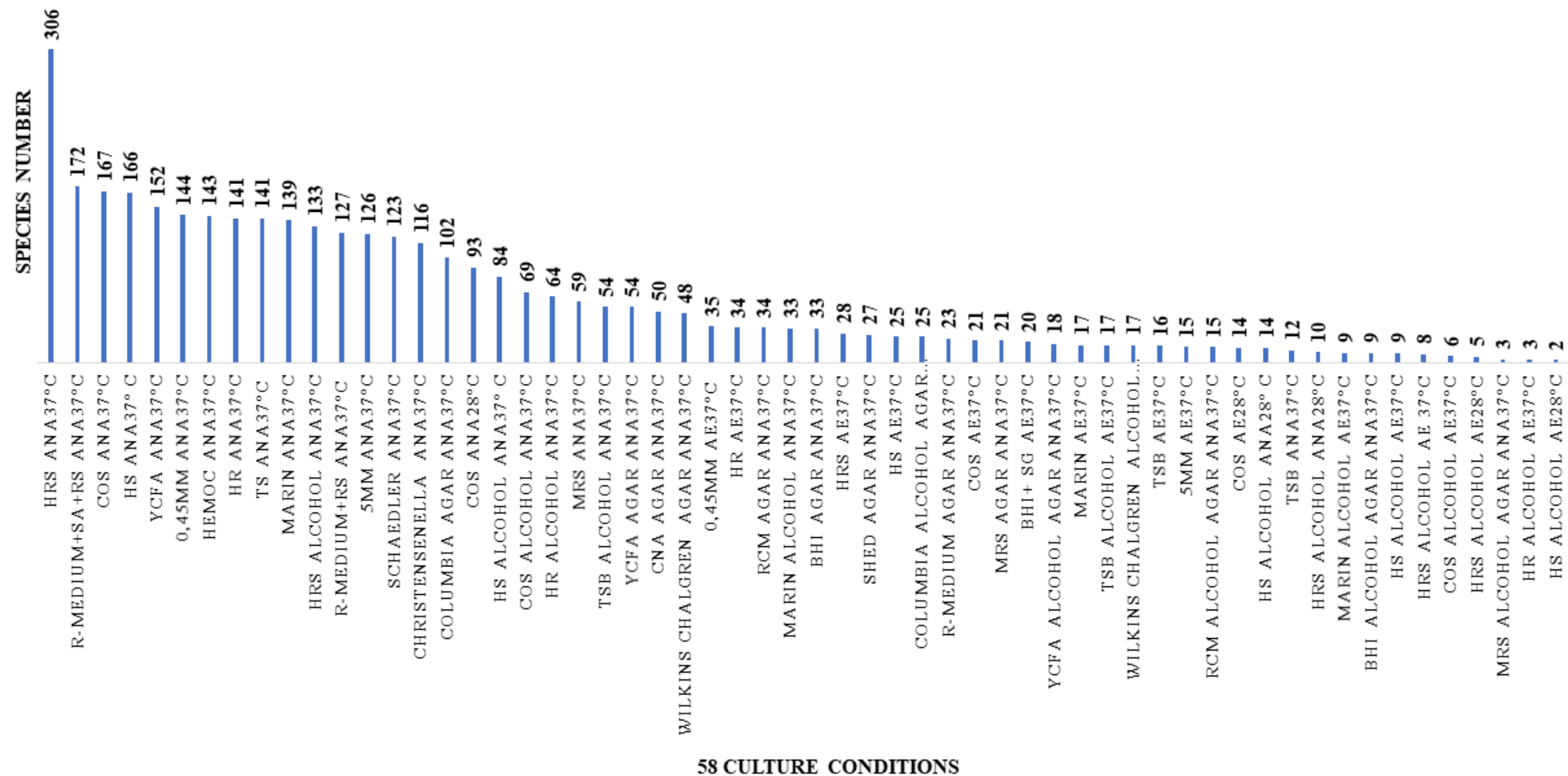
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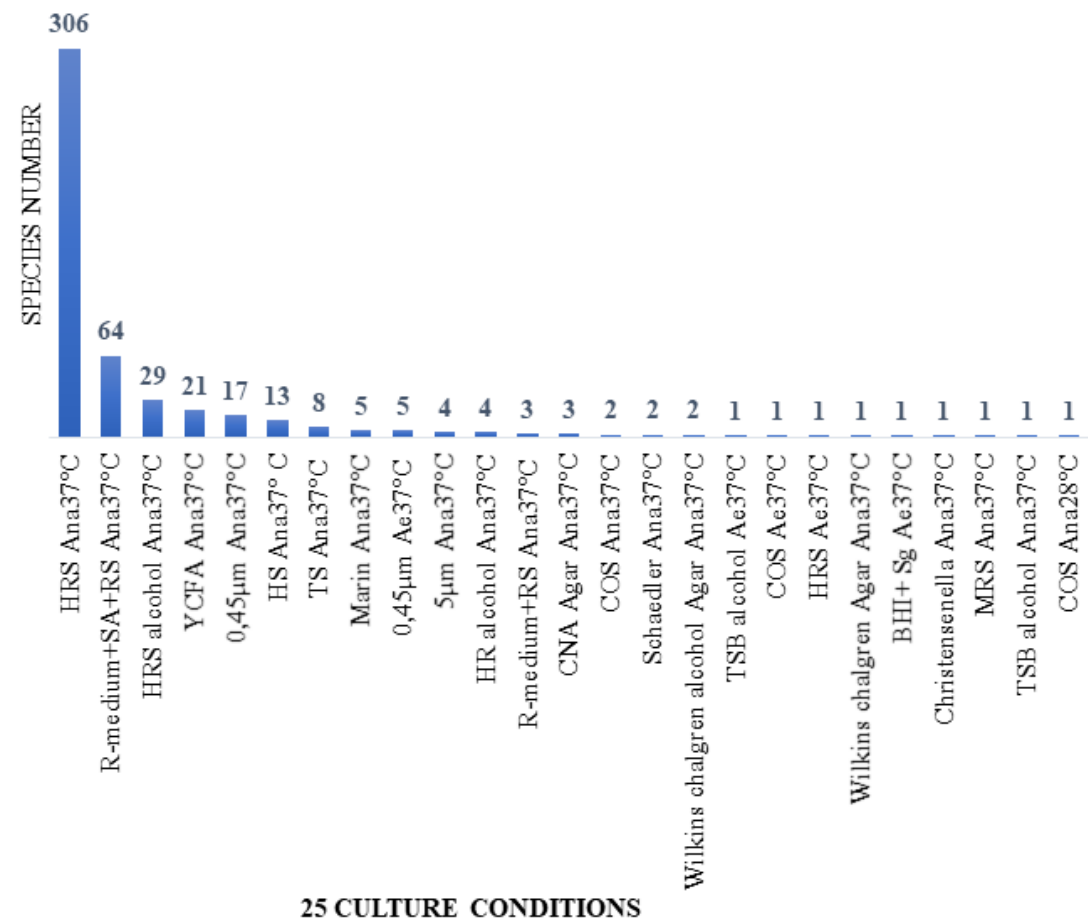
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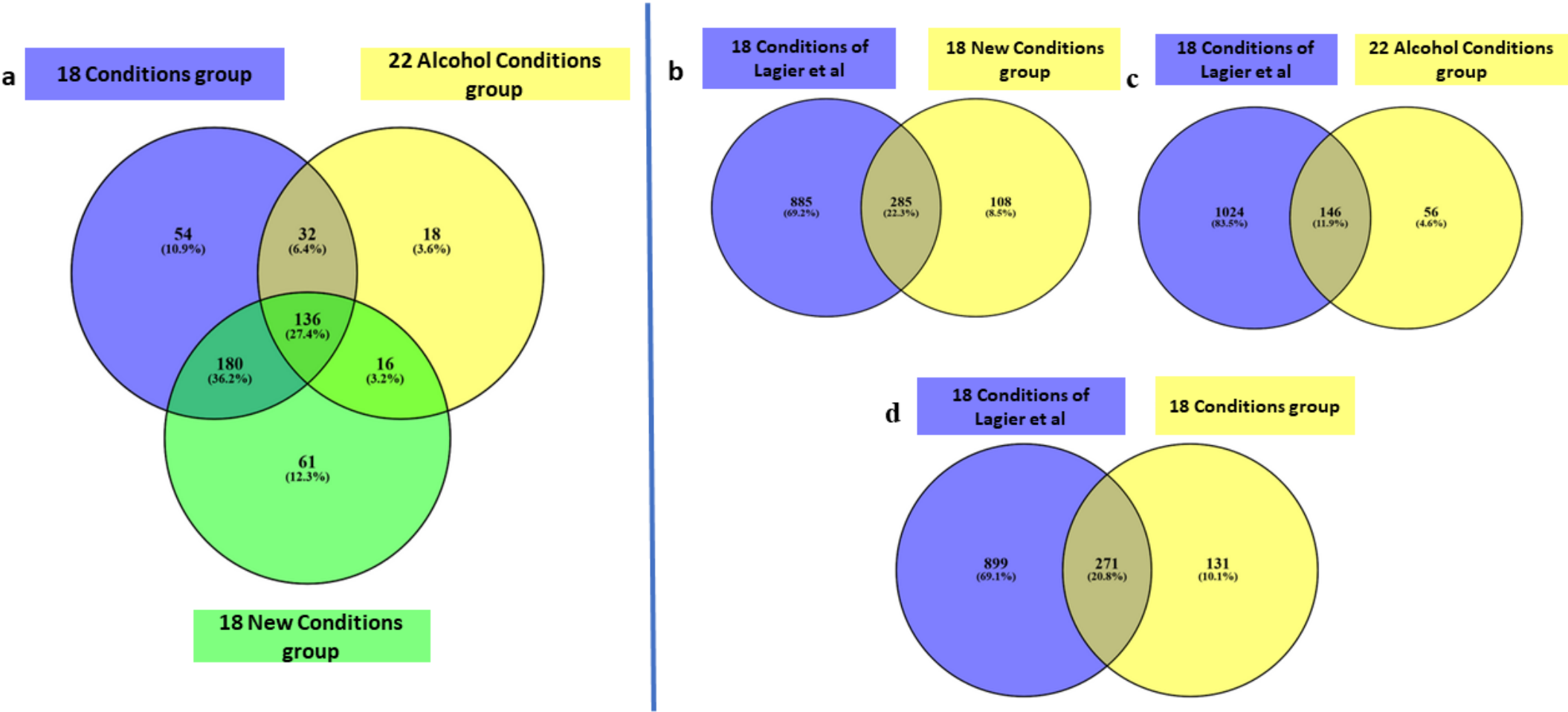
Supplementary Fig S1. Classification of culture conditions according to their profitability in number of isolated species



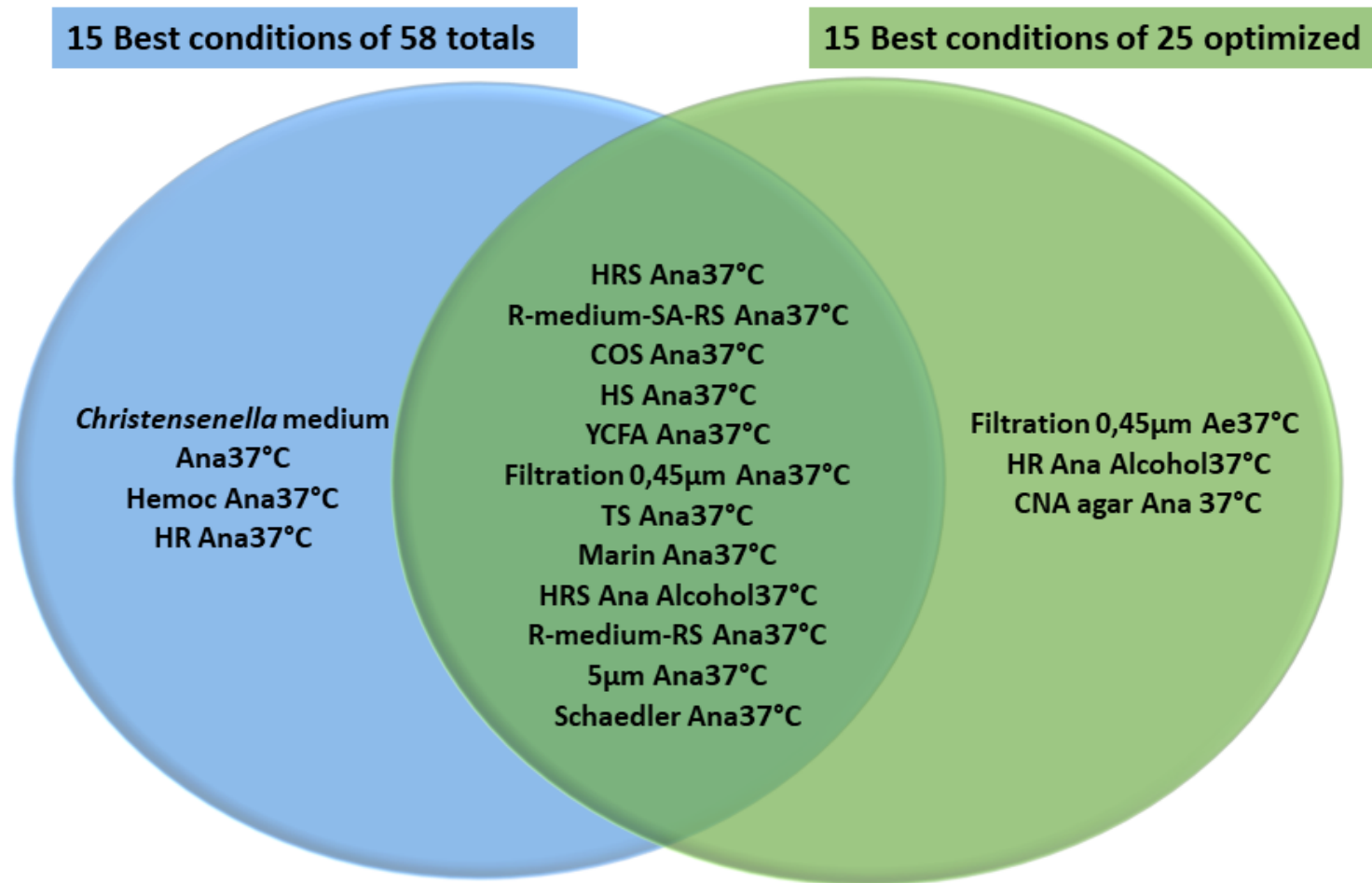
Supplementary Fig S2. Classification of culture conditions according to their ability to add the greatest number of previously non-isolated species by HRS Ana 37°C condition.



Supplementary Fig S3. Comparison of the bacterial species between the 3 groups of culture conditions: 18 previously selected conditions group, 18 new conditions group and 22 alcohol conditions group **(a)**. Each group was distinctively compared to the list of species isolated from the human gut by culturomics, established by Lagier et al **(b)**, **(c)**, **(d)**.



Supplementary Fig. S4: Comparison between the 15 best conditions of 58 totals conditions tested and the 15 best conditions of 25 conditions optimized in this study.



Supplementary table S1: 58 Culture Conditions use in this study.

18 culturomics standard conditions	
1-	Preincubation of stool preparation in anaerobic blood culture bottle (Hemoc Ana37°C)
2-	Preincubation of stool preparation in aerobic blood culture bottle with 5ml of rumen fluid (HR Ae37°C)
3-	Preincubation of stool preparation in anaerobic blood culture bottle with 5ml of rumen fluid (HR Ana37°C)
4-	Preincubation of stool preparation in aerobic blood culture bottle with 5ml of sheep blood (HS Ae37°C)
5-	Preincubation of stool preparation in anaerobic blood culture bottle with 5ml of sheep blood (HS Ana37° C)
6-	Preincubation of stool preparation in aerobic blood culture bottle with 5ml of rumen fluid and 5ml of sheep blood (HRS Ae37°C)
7-	Preincubation of stool preparation in anaerobic blood culture bottle with 5ml of rumen fluid and 5ml of sheep blood (HRS Ana37°C)
8-	Preincubation of stool preparation filtered at 5µm in aerobic blood culture bottle (5µm Ae37°C)
9-	Preincubation of stool preparation filtered at 5µm in anaerobic blood culture bottle (5µm Ana37°C)
10-	Preincubation of stool preparation after thermic shock at 80°C during 20min in anaerobic blood culture bottle (TS Ana37°C)
11-	Preincubation of stool preparation in COS liquid medium with 5% sheep blood in aerobic condition (Cos Ae37°C)
12-	Preincubation of stool preparation in COS liquid medium with 5% sheep blood in anaerobic condition (Cos Ana37°C)
13-	Preincubation of stool preparation in COS liquid medium with 5% sheep blood in aerobic condition (Cos Ae28°C)
14-	Preincubation of stool preparation in COS liquid medium with 5% sheep blood in anaerobic condition (Cos Ana28°C)
15-	Preincubation of stool preparation in Marine broth in aerobic condition (Marin Ae37°C)
16-	Preincubation of stool preparation in Marine broth in anaerobic condition (Marin Ana37°C)
17-	Preincubation of stool preparation in Trypticase Soy Broth medium in aerobic condition (TSB Ae37°C)
18-	Preincubation of stool preparation in Brain Heart Infusion broth with 5% sheep blood in aerobic condition (BHI+ Sg Ae37°C)
New culture media introduced	
1-	Preincubation of stool preparation in YCFA medium in anaerobic condition (YCFA Ana37°C)
2-	Preincubation of stool preparation in Christensenella medium in anaerobic condition (Christensenella Ana37°C)
3-	Preincubation of stool preparation in MRS medium in anaerobic condition (MRS Ana37°C)
4-	Preincubation of stool preparation in Schaedler medium in anaerobic condition (Schaedler Ana37°C)
5-	Preincubation of stool preparation in R-medium and lamb serum with 5ml of rumen fluid and 5ml of sheep blood in anaerobic condition (R-medium+SA+RS Ana37°C)
6-	Preincubation of stool preparation in R-medium with 5ml of rumen fluid and 5ml of sheep blood in anaerobic condition (R-medium+RS Ana37°C)
7-	Preincubation of stool preparation filtered at 0,45µm in aerobic blood culture (0,45µm Ae37°C)
8-	Preincubation of stool preparation filtered at 0,45µm in anaerobic blood culture bottle (0,45µm Ana37°C)
9-	Preincubation of stool preparation in Trypticase Soy Broth medium in anaerobic condition (TSB Ana37°C)
10-	Direct culture of stool preparation in CNA Agar medium in anaerobic condition (CNA Agar Ana37°C)
11-	Direct culture of stool preparation in R-medium Agar in anaerobic condition (R-medium Agar Ana37°C)
12-	Direct culture of stool preparation in Schaedler Agar medium in anaerobic condition (Shed Agar Ana37°C)

13- Direct culture of stool preparation in YCFA Agar medium in anaerobic condition (YCFA Agar Ana37°C)
14- Direct culture of stool preparation in Reinforced clostridial Agar medium in anaerobic (RCM Agar Ana37°C)
15- Direct culture of stool preparation in Wilkins chalgren Agar medium in anaerobic condition (Wilkins chalgren Agar Ana37°C)
16- Direct culture of stool preparation in Brain-heart infusion Agar medium in anaerobic condition (BHI Agar Ana37°C)
17- Direct culture of stool preparation in Columbia Blood Agar medium in anaerobic condition (Columbia Agar Ana37°C)
18- Direct culture of stool preparation in MRS Agar medium in anaerobic condition (MRS Agar Ana37°C)
22 Alcohol condition
1- Preincubation of stool preparation in aerobic blood culture bottle with 5ml of rumen fluid (HR alcohol Ae37°C)
2- Preincubation of stool preparation in anaerobic blood culture bottle with 5ml of rumen fluid (HR alcohol Ana37°C)
3- Preincubation of stool preparation in aerobic blood culture bottle with 5ml of sheep blood (HS alcohol Ae37°C)
4- Preincubation of stool preparation in anaerobic blood culture bottle with 5ml of sheep blood (HS alcohol Ana37° C)
5- Preincubation of stool preparation in aerobic blood culture bottle with 5ml of sheep blood (HS alcohol Ae28°C)
6- Preincubation of stool preparation in anaerobic blood culture bottle with 5ml of sheep blood (HS alcohol Ana28° C)
7- Preincubation of stool preparation in aerobic blood culture bottle with 5ml of rumen fluid and 5ml of sheep blood (HRS alcohol Ae 37°C)
8- Preincubation of stool preparation in anaerobic blood culture bottle with 5ml of rumen fluid and 5ml of sheep blood (HRS alcohol Ana37°C)
9- Preincubation of stool preparation in aerobic blood culture bottle with 5ml of rumen fluid and 5ml of sheep blood (HRS alcohol Ae28°C)
10- Preincubation of stool preparation in anaerobic blood culture bottle with 5ml of rumen fluid and 5ml of sheep blood (HRS alcohol Ana28°C)
11- Preincubation of stool preparation in COS liquid medium with 5% sheep blood in aerobic condition (Cos alcohol Ae37°C)
12- Preincubation of stool preparation in COS liquid medium with 5% sheep blood in anaerobic condition (Cos alcohol Ana37°C)
13- Preincubation of stool preparation in Marine broth in aerobic condition (Marin alcohol Ae37°C)
14- Preincubation of stool preparation in Marine broth in anaerobic condition (Marin alcohol Ana37°C)
15- Preincubation of stool preparation in Trypticase Soy Broth medium in aerobic condition (TSB alcohol Ae37°C)
16- Preincubation of stool preparation in Trypticase Soy Broth medium in anaerobic condition (TSB alcohol Ana37°C)
17- Direct culture of stool preparation in YCFA Agar medium in anaerobic condition (YCFA alcohol Agar Ana37°C)
18- Direct culture of stool preparation in Reinforced clostridial Agar medium in anaerobic (RCM alcohol Agar Ana37°C)
19- Direct culture of stool preparation in Wilkins chalgren Agar medium in anaerobic condition (Wilkins chalgren alcohol Agar Ana37°C)
20- Direct culture of stool preparation in Brain-heart infusion Agar medium in anaerobic condition (BHI alcohol Agar Ana37°C)
21- Direct culture of stool preparation in Columbia Blood Agar medium in anaerobic condition (Columbia alcohol Agar Ana37°C)
22- Direct culture of stool preparation in MRS Agar medium in anaerobic condition (MRS alcohol Agar Ana37°C)

Supplementary table S2: Detailed composition of the different culture conditions used in this study.

CULTURE MEDIUM	COMPOSITION
Blood culture bottle	Blood culture bottle BACT/ALERT Flacons FAN® Plus (Fastidious Antimicrobial Neutralization) by Bioré 40 ml of complex medium supplemented with absorbent polymeric beads Aerobic Ref. 410851 Anaerobic Ref. 410852
COS liquid medium 5% sheep blood	Yeast Extract. 5 g Peptone. 5 g Brain Heart Infusion 10 g NH ₄ CL. 1 g K ₂ HPO ₄ 0.3 g KCL. 0.3 g Ascorbic Acid: 1 g Sheep Blood: 5% Distilled Water..... 1000 ml
Marine broth	Peptone. 5 g Yeast Extract. 1 g Ferric Citrate..... 0.1 g Sodium Chloride..... 19.45 g Magnesium Chloride 8.8 g Sodium Sulfate. 3.24 g Calcium Chloride..... 1.8 g Potassium Chloride..... 0.55 g Sodium Bicarbonate. 0.16 g Potassium Bromide..... 0.08 g Strontium Chloride 34 mg Boric Acid. 22 mg Sodium Silicate..... 4 mg Sodium Fluoride 2.4 mg Ammonium Nitrate..... 1.6 mg Disodium Phosphate..... 8 mg Distilled Water..... 1000 ml

Trypticase Soy Broth	Tryptone (Pancreatic Digest of Casein)..... 17 g Soytone (Peptic Digest of Soybean).... 3 g Glucose (Dextrose).....2.5 g Sodium Chloride (NaCl)..... 5 g Dipotassium Phosphate (K ₂ HPO ₄)... 2.5 g Distilled Water.....1000 ml pH 7.3 ± 0.2
Brain Heart Infusion broth	Calf Brains, infusion from.200 g Beef Hearts, infusion from. 250 g Proteose Peptone. 10 g Dextrose..... 2 g NaCl..... 5 g Na ₂ HPO ₄ 2.5 g Distilled Water.....1000 ml pH 7.4 +/- 0.2 For solid media add agar to the composition
YCFA medium (modified)	Casitone10 g Yeast Extract... 2.50 g Glucose5 g MgSO ₄ x 7 H ₂ O.....45 mg CaCl ₂ x 2 H ₂ O... 90 mg K ₂ HPO ₄ ... 0.45 g KH ₂ PO ₄ ... 0.45 g NaCl0.9 g Resazurin... 1 mg NaHCO ₃ ... 4 g L-Cysteine-HCl1 g Hemin... 10 mg Volatile fatty acids: Acetic Acid... 1.90 ml Propionic Acid...0.70 ml iso-Butyric Acid... 90 µl n-Valeric Acid... 100 µl iso-Valeric Acid... 100 µl Vitamin solution:

	Biotin... 2 mg Folic Acid... 2 mg Pyridoxine-HCl... 10 mg Thiamine-HCl x 2 H2O ... 5 mg Riboflavin... 5 mg Nicotinic Acid... 5 mg D-Ca-pantothenate... 5 mg Vitamin B12... 0.10 mg p-Aminobenzoic Acid... 5 mg Lipoic Acid... 5 mg Distilled Water... 1000 ml pH to 6.7 -6.8 For solid media add agar to the composition
Christensenella medium	Meat filtrate 600 ml Casitone30 g/l Yeast extract... 5 g/l Na-resazurin solution (0.1% w/v)... 0,5 ml/l L-Cysteine-HCl x H2O 0,5 g/l Sodium carbonate(Na2CO3) 1,5 g/l K2HPO4... 5 g/l Meat filtrate... 400 ml D-Glucose..... 4 g/l Cellobiose 1 g/l Maltose 1 g/l Starch (soluble)..... 1 g/l
MRS-Lactobacillus medium	Proteose Peptone... 10 g Beef Extract. 10 g Yeast Extract. 5 g Dextrose..... 20 g Sorbitan Monooleate. 1 g Ammonium Citrate..... 2 g Sodium Acetate 5 g MnSO4 xH2O..... 0.05 g Na2HPO4. 2 g Distilled Water..... 1000 ml pH 6.5 +/-0.2

	For solid media add agar to the composition
Schaedler medium	Enzymatic Digest of Casein. 5.6 g Enzymatic Digest of Soybean Meal. 1 g Enzymatic Digest of Animal Tissue. 5 g Yeast Extract. 5 g Sodium Chloride..... 1.7 g Potassium Phosphate. 0.82 g Dextrose..... 5.82 g Tris (hydroxymethyl) Aminomethane 3 g Hemin. 0.01 g L-Cystine 0.4 g Distilled Water..... 1000 ml pH: 7.6 ± 0.2 at 25°C For solid media add agar to the composition
R-medium	Casein Hydrolysate..... 15 g Proteose Peptone... 15 g Yeast Extract... 10 g α-cétoglutarate... 2 g NaCl... 5 g Na2S 0.5 g Haemin... 0.1 g K2HPO4... 0,83g Glucose 10 g L-Cystéine 0,5 g Uric acid... 0.4 g Ascorbic acid..... 1 g Glutathion... 0.1 g Distilled Water..... 1000 ml pH : 7.5 For solid media add agar to the composition
	Casein Hydrolysate..... 15g Proteose Peptone... 15g Yeast Extract... 10g α-cétoglutarate... 2g NaCl..... 5g

R-medium and lamb serum	Na2S 0.5 g Haemin... .. 0.1 g K2HPO4... .. 0,83g Glucose 10g L-Cystéine 0,5g Uric acid 0.4g Ascorbic acid..... 1g Glutathion... ..0.1 g Lamb Serum... .. 150 ml Distilled Water..... 1000 ml pH : 7.5
CNA solid medium	Pancreatic Digest of Casein... .. 12 g Peptic Digest of Animal Tissue 5 g Yeast Extract... .. 3 g Beef Extract... .. 3 g Corn Starch... .. 1 g Sodium Chloride..... 5 g Colistin... .. 10 mg Nalidixic Acid... .. 10 mg Sheep Blood, defibrinated... ..5% Agar 13.5 g Distilled Water..... 1000 ml
Reinforced clostridial solid medium	Peptone 10 g Beef Extract..... 10 g Yeast Extract 3 g Dextrose..... 5 g Sodium Chloride..... 5 g Soluble Starch..... 1 g Cysteine HCl 0.5 g Sodium Acetate 3 g Agar 13.5 g Distilled Water..... 1000 ml pH : 6.8±0.2
	Tryptone. 10 g Gelatin Peptone. 10 g

Wilkins chalgren solid medium	Yeast Extract. 5 g Glucose 1 g Sodium Chloride..... 5 g L-Arginine 1 g Sodium Pyruvate. 1 g Menadione. 0.0005 g Haemin. 0.005 g Agar 10 g Distilled Water..... 1000 ml pH 7.1 +/-0.2.
Columbia Agar medium	Pancreatic Digest of Casein 12.0 g Peptic Digest of Animal Tissue 5.0 g Yeast Extract 3.0 g Beef Extract 3.0 g Corn Starch 1.0 g Sodium Chloride..... 5.0 g Agar 13.5 g Sheep Blood, Defibrinated 5 % pH 7.3 ± 0.2

- Agar was autoclaved separately before being added to the broths.
- Sheep rumen is transported from the slaughterhouse to our laboratory. The digestive contents of sheep stomachs are filtered successively through a funnel covered with a laboratory charlotte. The solid contents are discarded. The suspension will be centrifuged at 10,000 rpm for 90 minutes, the supernatant will be collected for 3 successive filtrations at 0.8µm, 0.45µm, 0.2µm. We thus obtain our rumen juice which can be stored at 4°C.

Supplementary table S3: Donor characteristics.

Donors	Size (m)	Weight (Kg)	Body mass index	Age (years)	Sex	Origin
Megagut 1	1,65	60	22	28	Female	French
Megagut 2	1,80	72	22,2	32	Male	Senegalese
Megagut 3	1,84	76	22,4	32	Male	Senegalese
Megagut 4	1,75	55	18	27	Female	Beninese
Megagut 5	1,79	95	29,6	32	Male	Cameroon
Megagut 6	1,7	69	23,6	30	Male	French
Megagut 7	1.65	70	25,7	27	Female	Algerian
Megagut 8	1.78	82	25,9	27	Male	Algerian

