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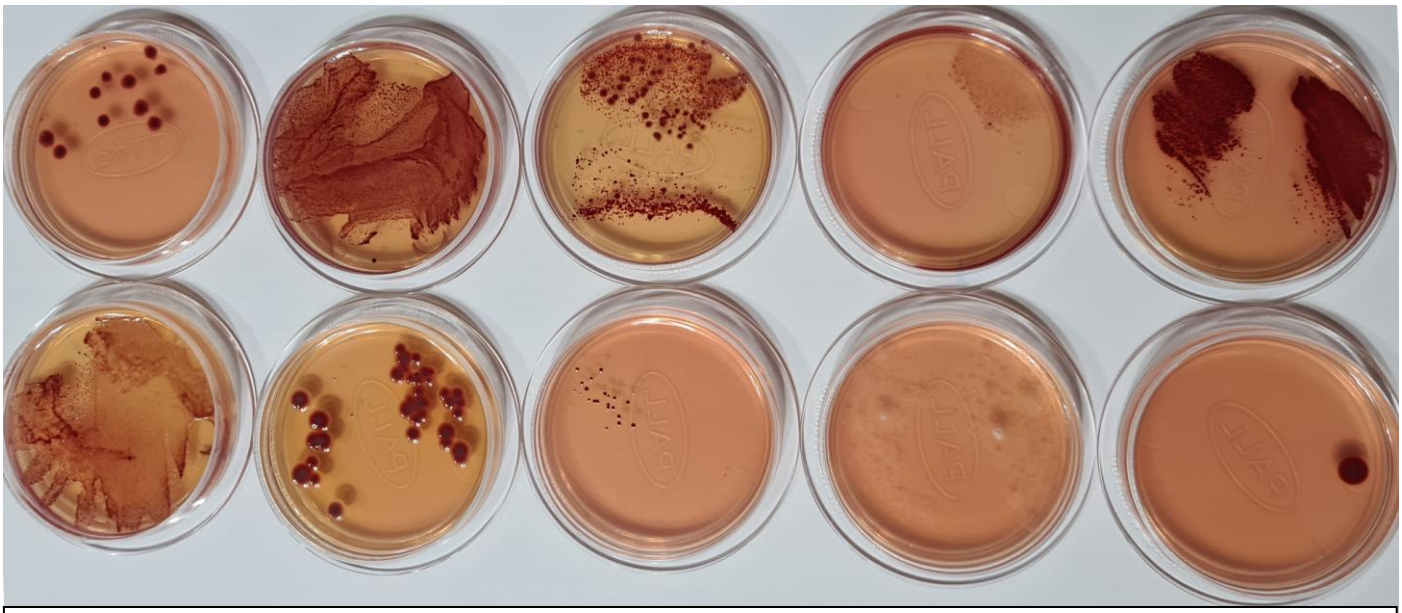
CROMOKIT® UNIVERSAL PATHOCHROM AGAR (CUP12A)

Universal chromogenic agar for selective isolation of the most typical pathogens in cosmetics

INTRODUCTION

EC Regulation No. 1223/2009 and GMP (ISO 22716) requires "cosmetic safety", that is, in microbiology: the absence of pathogens. A medium was needed that brought together the majority of pathogens that regularly cause market recalls (see RAPEX) of cosmetic products (there are 14, not just 4), with the consequent economic loss and media image of the brands involved. And MICROKIT has achieved it: CUP-12 Agar detects 2 of the 4 pathogens for which ISO Standards have been written (*E.coli* and *Pseudomonas aeruginosa*, not *Staphylococcus aureus* nor *Candida albicans*) and ALSO, it detects all the other (10) pathogens that cause the most market recalls at a Universal level!: *Aspergillus spp*, *Burkholderia cepacia* complex, Pathogenic coliforms (*Pluralibacter gergoviae*, *Klebsiella spp*, *Enterobacter spp*...), Pathogenic Enterobacteriaceae (*Proteus mirabilis*, *Salmonella spp*, *Serratia marcescens*...), Fecal Enterococci and *Pseudomonas putida*. Total, 12 of the 14 most typical pathogens in cosmetics. It was necessary to detect all of them, as we already indicated to the cosmetics industry: safety/security.



The 10 most typical cosmetic pathogens, detected in 36 hours by direct streaking of the enrichment broth in CUP12A. Above, from left to right: E.coli, P.aeruginosa, S.aureus, C.albicans, B.cepacia. Below, from left to right: Pluralibacter gergoviae, Klebsiella spp.pl, Enterococcus spp.pl, Aspergillus spp.pl, Salmonella spp.pl. Another 28 microorganisms not typical in cosmetics do not grow in this medium. It is not a differential medium with spectacular colors, but if a reddish colony grows, it is extremely likely that it is a typical cosmetic pathogen: you just have to confirm it!

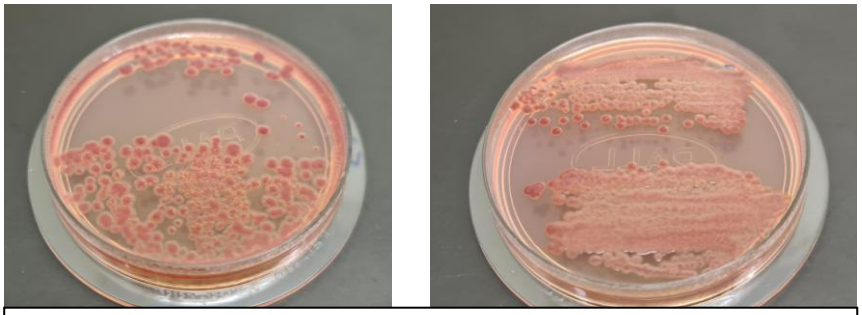
In this way, with only one isolation medium (CUP12A) after selective enrichment, any laboratory can specifically detect 12 of the 14 pathogens that it would never detect with only the 4 "ISO" media. But if the health authorities controlled a sample in this last situation, you would have a high probability of encountering a problematic recall of your batch, having released it to the market without analyzing more than the 4-5 most famous (but not most frequent) pathogens and ignoring the rest (which are contemplated in ISO 18415 of specified and unspecified microorganisms, but this causes much more work by having to identify all the colonies grown in TSA, almost always with harmless false positives, unlike CUP12A, where the innocuous ones do not grow). Aside from its value as an additional detector of the 10 most typical cosmetic pathogens that you are not currently looking for, imagine the amount of time, money, work, space, stoves, waste... that you will save when you replace 2 of the 4 media for *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* (those of the first two), for only one: CUP12A.

NOTE: Standards ISO 18416, ISO 22717, ISO 21150 and ISO 22718 ARE NOT mandatory under European legislation, as they are not published in the OJEU (DOUE), unlike the mandatory ISO 22716 GMP; They are only a summary of what has been done until now by the majority of laboratories. But different well-designed intercomparative cosmetic microbiology services are demonstrating, since before these Technical Standards were born, that they were very unrobust methods, that they were not really designed for inhibitory matrices with lethargic microbiota, such as cosmetics (and not substitutes without preservatives), and therefore caused numerous false negatives. And therefore NO ONE can ask their laboratory to anchor itself in an obsolete past, following these ISO Standards. Apart from the fact that they ignore the 10 emerging pathogens that are causing the most market recalls in the cosmetic world; because the health authorities do follow the legislation and seek the safety and security dictated by the legislation.

COMPOSITION

Nutritional factors	16.0
Various salts	7.10 g
Agar-agar	15.0 g
Mix of selective agents	c.s.
Chromogenic mixture	c.s.

(Formula per liter)
Final pH: adjust to 7.2 ± 0.2



Left: Pseudomonas putida in 18 h & Right: Burkholderia cepacia in 18-36h in CUP12A

PREPARATION

Dissolve 38.5 g of medium in 1 L of double-distilled water. Stir to homogenize, heating until boiling without stopping stirring. AUTOCLAVE at 121ºC for 15 minutes. Cool quickly in a bath to 45-50ºC, stirring gently. Do not remelt, dispense into sterile Petri dishes and allow to solidify. The color of the medium is softly orange. The plates, if they are well closed in self-sealing bags to avoid dehydration and contamination, can be stored for up to a month outside the refrigerator, in the dark, the airtight MICROKIT plates for up to 6 months, do not freeze!

FOR EXCLUSIVE USE IN LABORATORY. SHAKE THE POWDER CAN, BEFORE USE, TO HOMOGENIZE THE DENSITY GRADIENTS OF THE DIFFERENT COMPONENTS, POSSIBLY FORMED DURING STORAGE. KEEP THE JAR AND DRYPLATES WELL CLOSED, IN A DRY, COOL AND DARK PLACE. KEEP PREPARED PLATES, TUBES AND BOTTLES OUT OF THE REFRIGERATOR, IDEAL AT 18ºC, IN COMPLETE DARKNESS.

PRESENTATION

- DEHYDRATED MEDIA 500g Ref: DMT518
-DryPlates®-CUP12A Ref: DPPCUP12
- 55 mm Hermetic and stackable PLATES (PLAQUIS ®) Ref: PPL944
-90 mm PLATES Ref: ECOP08
-18 mL Tubes to prepare 1 of 90mm plate or 2-3 of 55mm plates with each tube Ref: TPL518
-100 mL Bottles to prepare 5 of 90 mm plates or 10-15 of 55 mm small plates with each bottle Ref: RPL518

QUALITY CONTROL

Made in our laboratory; It is prudent to repeat it in your laboratory whenever the conditions vary (more than 3 months without use, after disinfecting the laboratory, after storing at high temperatures, when it acquires strange appearances even though the theoretical expiration date on the label has not arrived,...) .
DEHYDRATED: Slightly pinkish cream powder PREPARED: Sterile, slightly orange
GROWTH CONTROL 48 h at approximately 35°C and immediate pre-identification:
Aspergillus niger brasiliensis WDCM 00053, Correct, cottony colonies
Burkholderia cepacia MKTA 25416, Correct, reddish colonies, Neogram negative, oxidase positive, ADH negative
Enterobacter aerogenes WDCM 00175, Correct, reddish colonies, Neogram negative, oxidase negative, indole negative
Enterococcus faecalis WDCM 00087, Correct, reddish colonies, Neogram positive, catalase negative
Escherichia coli WDCM 00012, Correct, reddish colonies, Neogram negative, oxidase negative, indole positive
Klebsiella pneumoniae WDCM 00206, Correct, reddish colonies, Neogram negative, oxidase negative, indole negative
Pluralibacter gergoviae MKTD 9245, Correct, reddish colonies, Neogram negative, oxidase negative, indole negative
Proteus mirabilis WDCM 00023 Correct, reddish colonies, Neogram negative, oxidase negative, indole negative
Pseudomonas aeruginosa WDCM 00025, Correct, reddish colonies, Neogram negative, oxidase +, acetamide +, ADH +
Pseudomonas putida WDCM 00117, Correct, reddish colonies, Neogram negative, oxidase positive
Salmonella enterica typhimurium WDCM 00031, Correct, reddish colonies, Neogram negative, oxidase negative, indole negative
VALIDATION report with 20 different COSMETICS. Furthermore, in 9 months of analyzing cosmetics in our lab, we have not found a single false positive in CUP12Agar that would make us lose confirmation time.

INSTRUCTIONS FOR USE AND READING OF RESULTS

Strike the enriched broth on the surface of the prepared plate (Ex: LPT Neutralizing Broth, Eugon New Broth, Letheen Modif Broth, D/E Broth...). If desired, you can streak one half of the 10 µL loop plate and another on the other half 1 µL loop plate to increase the probability of isolating pure colonies. Or better, one streak from the enriched dilution (-1) and another from the (-2). Generally after 36-48 h of enrichment in the aforementioned broths, any contaminant is well detected with a 10 µL loop.
Incubate CUP12A at 35ºC for 36-48h.
Look for typical stretch marks or colonies (reddish: pink, orange, fuchsia, purple...), which will almost always be typically cosmetic pathogens, so the medium serves as a prior warning. If desired, you can confirm which strains they are, by re-sowing in the appropriate media and/or with immediate appropriate tests (Neogram KIN001, Oxidase KOT050, Catalase KMT299, Indol SBH056, ADH TPLADH, Acetamide TPL113 + Nessler SMT007, Coagulase KWD094) and, once oriented with these on the group to which the colonies belong, use galleries or other additional tests.
Logically, looking for 14 pathogens instead of 4, will require more confirmation work, but we will be able to ensure what the legislation really asks for us: the absence of pathogens specific to cosmetics (Especially if we enrich in LPT Neutralizing Broth from MICROKIT, as has been demonstrated in intercomparison proficiency test schemes).

RESULTS of pathogens in CUP-12 AGAR after passing each strain through cosmetics and 48 hours in LPTN Broth at 35ºC, at different times (12-48 hours)		12 h at 35ºC	18h at 35ºC	36 h at 35ºC	48h at 35ºC
WDCM 00053	*Aspergillus niger brasiliensis*	+	+	+	+
DSMZ 50181	*Burkholderia cepacia*			+	+
ATCC 25416	*Burkholderia cepacia*	+	+	+	+
ATCC BAA-245	*Burkholderia cenocepacia*		+	+	+
ATCC BAA-247	*Burkholderia multivorans*		+	+	+
WDCM 00054	*Candida albicans* *		+	+	+
WDCM 00006	*Citrobacter freundii*		+	+	+
WDCM 00175	*Enterobacter aerogenes*	+	+	+	+
WDCM 00087	*Enterococcus faecalis*	+	+	+	+
WDCM 178	*Enterococcus faecium*		+	+	+
DSMZ 20160	*Enterococcus hirae*		+	+	+
WDCM 00013	*Escherichia coli*				+
WDCM 00196	*Escherichia coli*	+	+	+	+
WDCM 00090	*Escherichia coli*				+
WDCM 00012	*Escherichia coli*		+	+	+
WDCM 00097	*Klebsiella aerogenes (Raoultella planticola)*	+	+	+	+
DSMZ 5175	*Klebsiella oxytyoca*	+	+	+	+
WDCM 00206	*Klebsiella pneumoniae (K.variicola)*	+	+	+	+
MKTS-BCN1	*Pantoea agglomerans salvaje*			+	+
DSMZ 9245	*Pluralibacter gergoviae*	+	+	+	+
WDCM 00023	*Proteus mirabilis*		+	+	+
WDCM 00025	*Pseudomonas aeruginosa*	+	+	+	+
WDCM 00117	*Pseudomonas putida*		+	+	+
WDCM 00030	*Salmonella enterica ssp enteritidis*		+	+	+
WDCM 00031	*Salmonella enterica ssp typhimurium*				+
DSMZ 30121	*Serratia marcescens*				+
MKTS-SH01	*Shigella flexneri salvaje*				+
WDCM 00034	*Staphylococcus aureus*				+
WDCM 00131	*Staphylococcus aureus.*	+	+	+	+
WDCM 00032	*Staphylococcus aureus* *				-
PECJJT	*Staphylococcus hominis salvaje*		+	+	+

* Some strains of *St.aureus* and *Candida albicans* do not grow well on CUP-12 Agar: do not substitute the media for these two pathogens with this almost-universal medium, but you can substitute the other media you use for the other pathogens and so on will definitely increase the range of pathogens detected

Add CUP-12A to the 4-5 media you already use for the most classic pathogens (or at least to the Staphylococcus and Candida media), and you will also be able to detect the emerging pathogens that, until now, you were not looking for, and legislation requires its absence.

DOUBLE BENEFIT: DETECT ALL TYPICAL PATHOGENS OF COSMETICS AND SAVE 2-3 ADDITIONAL CULTURE MEDIA, plus the time, money, work, space, stoves, waste... that this costs you. Additional advantage: CUP-12 Agar is much more economical than separately ordering the media it replaces for *E.coli, Ps.aeruginosa* and *Burkholderia cepacia*, even from the cheapest brands. And only 38.5 g/L is needed, so 1 bottle of 500 g is enough to make 13 L, that is, 722-867 90 mm plates, 1,083 55 mm plates or no less than 2,167 airtight and stackable PLAQUIS® from MICROKIT (Ref: VDA002).

12 of the 14 cosmetic pathogens in one medium? CUP-12 AGAR is almost a miracle! a tribute to creativity when several multidisciplinary experts come together with the common objective of helping to comply with authentic legislation. Fly your laboratory into the 21st Century!

The concordance between the results of the different media for pathogens and CUP12A is spectacular (99.17% efficiency) both in its sensitivity component and in its specificity component, that is: there are practically no false positives that make us waste time in confirming non-pathogenic microorganisms, nor false negatives that make us lose faith in CUP12A.

The end user is solely responsible for the destruction of the organisms that have developed during analysis, according to current environmental legislation: Autoclave before disposing of it in the trash.

Medium designed and validated by MICROKIT between 2-2021 and 4-2023. Manufactured in the EU exclusively by MICROKIT, under ISO 9001, ISO 11133 and GMPs, since February 14, 2022. Reviewed on April 10, 2024.

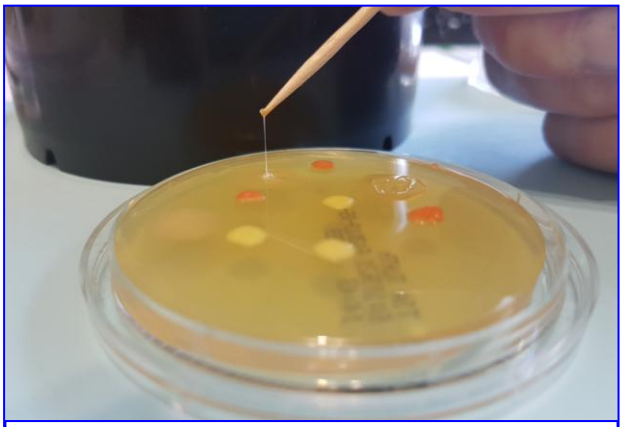
Dichotomous key to guide you in confirming CUP-12A colonies
Typical pathogens of cosmetic product recalls distinguishable from CUP-12A with only 6 reagents:

Cottony colony: possible *Aspergillus fumigatus (green), A.flavus (yellow)* or other pathogenic Aspergillus
Colony different:

Colony big and white in SDA, dun in Biggy, pink in RB, that emits a yeasty smell: possible *Candida albicans*
Colony different:

Colony Neogram positive (does not filaments):
- Catalase negative (no effervescence): possible *Enterococcus faecalis*,
- Catalase positive (effervescence), coagulase positive: very probable *Staphylococcus aureus*

Colony Neogram negative (filaments):
- Oxidase positive (turns to dark blue): Non Fermenters
  - ADH negative (yellow or orange): probable *Burkholderia cepacia*,
  - ADH positive (turns to red): *Pseudomonas aeruginosa* (moreover is Acetamide + and fluorescent in KingB Agar)
- Oxidase negative (Not turns to blue): Fermenters
  - Indol positive at 44ºC: *Escherichia coli*
  - Indol positive at 37ºC: *Klebsiella spp, Citrobacter spp, Escherichia coli*
  - Indol negative:
    - Enterobacteria non-coliforms (does not ferments lactose): *Proteus mirabilis, Salmonella spp, Pantoea spp, Shigella spp*
    - Coliforms (ferments lactose): *Klebsiella spp, Enterobacter spp, Pluralibacter gergoviae, Salmonella spp , Serratia spp, Citrobacter spp.*



Neogram: Filaments (Gram negative); if not: Gram positive

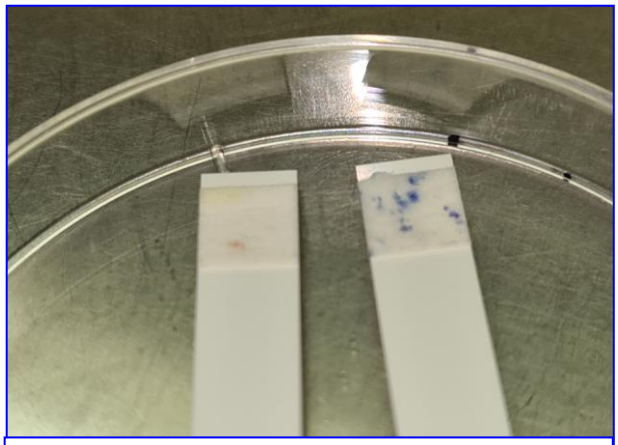
INDICATED TEST AND DECISION TIME:

IMMEDIATE:

Neogram (Ref: KIN001)	20 seconds
Catalase (Ref: KMT299)	2 seconds
Oxidase (Ref: KOT050)	30 seconds
Coagulase (Ref: KWD094)	2 minutes

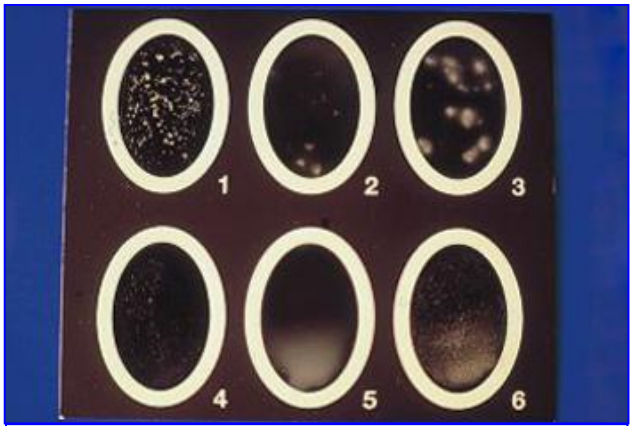
NO IMMEDIATE:

ADH (Ref: TPLADH)	18 hours
Indol Kovacs (Ref: SBH056) en Triptophan Water (Ref: TPL034)	18 hours
Lactose c/D (Ref: TPL020)	18 hours



Left: Oxidase negative Right: Oxidase positive: dark blue

Catalase positive: colony effervescence



1, 4: Coagulase + strong & weak; 2,3: clumps of poorly dissolved colonies; 5,6: milky bottom, coagulase -