Brief Report

SCA Medium: A New Culture Medium for the Isolation of All Candida auris Clades

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Abstract: Candida auris is an emerging multidrug-resistant yeast causing nosocomial infections and associated with high mortality in immunocompromised patients. Rapid identification and characterization are necessary for diagnosis and containing its spread. In this study, we present a selective culture medium for all C. auris clades. This medium is sensitive with a limit of detection ranging between 10^1 and 10^2 CFU/mL. The 100% specificity of SCA (specific C. auris) medium is confirmed on a set of 135 Candida strains, 50 bacterial species and 200 human stool samples. Thus, this medium specifically selects for C. auris isolation from clinical samples, allowing the latter to study its phenotypic profile.

Keywords: Candida; Candida auris; culture; emerging fungus; isolation; specific medium

1. Introduction

Candida auris is an emerging multidrug-resistant pathogen that was first isolated in 2009 [1] and is now known to have four geographical clades: South Asia: India; East Asia: Japan; Southern Africa: South Africa; and South America: Venezuela [2]. Recently, a potential fifth clade (Clade V) has been described in Iran [3]. C. auris is a biofilm-forming, halotolerant, thermo-resistant yeast [1,4] that can grow at temperatures ranging between 30 °C and 42 °C and can tolerate up to 10% salinity [5]. This promotes the ability of C. auris to colonize various medical equipment, plastic surfaces, and nosocomial environments [2–4], rendering C. auris an invasive, powerful pathogen. In addition, C. auris is highly resistant to different classes of antifungal agents such as azoles, amphotericin B and echinocandins [6] and is associated with significant mortality, especially in immunocompromised patients with multiple comorbidities such as diabetes mellitus, renal failure, and cardiovascular disease [2,4]. Thus, its rapid identification and characterization are necessary to optimize clinical outcomes and to attempt to contain its nosocomial and worldwide spread.

The diagnosis of C. auris is challenging. The vast majority of commercial, readily available diagnostics, such as VITEK, API20C-AUX, Auxa-Color 2, BD Phoenix and MicroScan are misleading. C. auris is misidentified as other Candida species, namely Candida famata, Candida haemulonii, Candida duobushaemulonii, Candida sake, Candida lusitaniae and Candida guilliermondii among others [7,8]. This often leads to delays in appropriate management and treatment. However, the use of Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) is promising [7,8], since the spectrum of C. auris is available in the reference database. In addition, the user can carefully select, “hand-pick”, and isolate the corresponding identified colonies from other colonies growing on a plate. Moreover, the molecular identification and characterization of C. auris is well
developed [9], and the use of some real-time PCR-targeting \textit{C. auris} and sequencing of the ITS region is promising [9–12]. In 2017, a modified Sabouraud medium was suggested by Welsh et al. for a specific isolation of \textit{C. auris} [5]. However, some \textit{Candida} isolates were still misidentified [7,8]. Thus, our aim was to develop a specific medium only selective for all \textit{C. auris} clades from clinical samples.

2. Materials and Methods

We collected 135 fungal strains (29 different species) [13–15] (Table 1) and cultured them on the modified Sabouraud broth suggested by Welsh et al. that contains 20 g/L Mannitol as a carbon source (in order to inhibit \textit{C. glabrata} growth) [5] in both liquid and solid-phase media. For the liquid phase, turbidity of the broth was measured by spectrophotometry at 24, 48, and 72 h of incubation at 40 °C. This served to control the growth of each tested strain. For the solid phase, we added autoclaved bacterial agar (15 g/L) and adjusted for a pH = 7. Each colony was streaked directly on the solid agar or the liquid inoculum.

| Type                      | Strain/Sample | Nb | Source | Origin      | Identification          | qPCR \textit{C. auris} |
|---------------------------|---------------|----|--------|-------------|-------------------------|--------------------------|
| Gram-positive bacteria    | \textit{Bacillus cereus} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Corynebacterium amycolatum} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Corynebacterium jeikeium} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Corynebacterium propinquum} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Corynebacterium striatum} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Enterococcus faecalis} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Enterococcus faecium} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Micrococcus luteus} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Staphylococcus aureus} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Staphylococcus capitis} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Staphylococcus cohnii} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Staphylococcus epidermidis} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Staphylococcus haemolyticus} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Staphylococcus lugdunensis} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
|                           | \textit{Staphylococcus pasteuri} | 1  | Clinical | Marseille, France | MALDI-TOF-MS | Not tested              |
| Type                        | Strain/Sample        | Nb | Source   | Origin             | Identification      | qPCR C. auris (Ibrahim et al., 2021) |
|-----------------------------|----------------------|----|----------|--------------------|---------------------|---------------------------------------|
|                             |                      |    |          |                    |                     |                                       |
| Gram-positive bacteria      |                      |    |          |                    |                     |                                       |
| Staphylococcus saprophyticus | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Staphylococcus simulans     | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Staphylococcus warneri      | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Streptococcus agalactiae    | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Streptococcus dysgalactiae  | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Streptococcus equinus       | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Streptococcus mitis         | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Gram-negative bacteria      |                      |    |          |                    |                     |                                       |
| Achromobacter xylosoxidans  | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Acinetobacter baumannii     | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Bacteroides fragilis        | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Citrobacter braakii         | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Citrobacter freundii        | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Citrobacter koseri          | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Enterobacter aerogenes      | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Enterobacter asburiae       | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Enterobacter cloacae        | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Enterobacter kobei          | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Escherichia coli            | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Haemophilus influenzae      | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Haemophilus parainfluenzae  | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Hafnia alvei                | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Klebsiella oxytoca          | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| Klebsiella pneumoniae       | 1 Clinical           | 1  | Clinical | Marseille, France  | MALDI-TOF-MS        | Not tested                            |
| **Subtotal**                | 25                   |    |          |                    |                     |                                       |
Table 1. Cont.

| Type          | Strain/Sample      | Nb  | Source    | Origin     | Identification       | qPCR C. auris (Ibrahim et al., 2021) |
|---------------|--------------------|-----|-----------|------------|----------------------|---------------------------------------|
| Moraxella catarrhalis | 1 Clinical |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Morganella morganii     | 1 Clinical |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Pasteurella multocida   | 1 Clinical |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Proteus mirabilis       | 1 Clinical |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Proteus vulgaris        | 1 Clinical |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Providencia stuartii    | 1 Clinical |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Pseudomonas aeruginosa  | 1 Clinical |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Raoultella ornithinolytica | 1 Clinical |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Stenotrophomonas maltophilis | 1 Clinical |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| **Subtotal**            |        | **25** |            |            |                      |
| **Yeast**               |        |       |            |            |                      |
| Candida albicans       | 73 Clinical |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida glabrata       | 8 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida krusei         | 4 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida parapsilosis   | 6 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida lusitaniae     | 3 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida tropicalis      | 6 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida zelanooides    | 1 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida lipolytica     | 1 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida inconspicua    | 1 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida intermedia     | 1 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida guilliermondii | 3 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida bracarensis    | 1 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida utilis         | 1 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida bovina         | 1 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida dubliniensis   | 2 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
| Candida norvegensis    | 1 Clinical  |       | Marseille, France | MALDI-TOF-MS | Not tested           |
We then cultured all *C. auris* (*n* = 7) and *Candida tropicalis* (*n* = 6) (see results) strains on the different culture media used in our diagnostic laboratories. The media used for culturing yeasts were: Sabouraud Dextrose Agar, CHROMagar *Candida* and Buffered Charcoal Yeast Extract. The media used for culturing bacteria were: Chocolate agar, Tryptic Soy agar, Columbia agar, Mannitol Salt agar, MacConkey agar (BioMérieux, Marcy-l’Etoile, France) and LB|MR (Lucie Bardet and Jean-Marc Rolain) medium [16].

Finally, we cultured this set of 13 strains (i.e., *C. tropicalis* and *C. auris*) on different combinations of the Welsh et al. broth and MacConkey (MCK) agar, a selective medium for Gram-negative bacteria (see results and discussion) (Table 2) [17]. We prepared several media with varying concentrations of bile salts (1.5 g/L, 1 g/L, 0.75 g/L) and/or crystal violet (0.5 mg/L) (used as inhibitors in MCK) [17] to the initial broth composition of Welsh et al. in solid and liquid phase with shaking (300 rpm) at 40 °C (Table 2).
Table 2. Growth results of *C. auris* (seven strains from four clades) and *C. tropicalis* (*n* = 6) tested strains according to crystal violet and bile salts concentrations for each condition. 5 g of pancreatic digest of casein, 5 g of peptic digest of animal tissue, 100 g NaCl, 20 g of Mannitol, 50 mg/L chloramphenicol and 50 mg/L gentamicin were added.

| Crystal Violet | Bile Salts | *C. auris* Clade I | *C. auris* Clade II | *C. auris* Clade III | *C. auris* Clade VI | *C. tropicalis* |
|---------------|-----------|--------------------|--------------------|---------------------|---------------------|----------------|
| 0 mg/L        | 0 g/L     | ++                 | ++                 | ++                  | ++                  | ++             |
| 0 mg/L        | 0.5 g/L   | ++                 | ++                 | ++                  | ++                  | ++             |
| 0 mg/L        | 1 g/L     | ++                 | ++                 | ++                  | ++                  | ++             |
| 0 mg/L        | 1.5 g/L   | ++                 | ++                 | ++                  | ++                  | ++             |
| 0.5 mg/L      | 0 g/L     | ++                 | ++                 | ++                  | ++                  | ++             |
| 0.5 mg/L      | 0.5 g/L   | ++                 | ++                 | ++                  | ++                  | ++             |
| 0.5 mg/L      | 1 g/L     | ++                 | ++                 | ++                  | ++                  | ++             |
| 0.5 mg/L      | 1.5 g/L   | ++                 | ++                 | ++                  | ++                  | ++             |

To study the specificity of our designated medium, we cultured for 3 days at 40 °C a panel of various Gram-positive and Gram-negative bacteria (*n* = 50), different *C. auris* clades (DSM 21092 and 6 strains that were kindly provided by Dr. Jacques F Meis, Canisius Wilhemina Hospital, Department of Medical Microbiology and Infectious Diseases, the Netherlands) [9,18–20]. All *C. auris* strains were isolated from blood cultures except JCM 15,448 (Clade II), which is from the external ear canal. We tested also other *Candida* strains (including the closest *Candida* species to *C. auris* (*C. haemulonii* and *C. duobushaemulonii*) (directly streaked on the agar) [2,4]. In addition, we tested 200 fecal samples that were negative by real-time PCR for *C. auris*. These samples were collected at the Marseille Hospital (AP-HM, Assistance Publique-Hôpitaux Marseille) from routine laboratory diagnostics (Table 1). Given that this work did not involve human body/tissues or use clinical data from patients, and according to French law (Loi no 2012–300 of 5 March 2012 and Décret no 2016–1537 of 16 November 2016 published in the ‘Journal Officiel de la République Française’), neither institutional ethical approval nor individual patient consent was required for this non-invasive study.

All patients routinely sign an approval that the samples they submit for testing at AP-HM labs may be used in research. Each stool sample was enriched in Tryptic soy broth (TSB) for 3 days at 37 °C. Then, a total of 0.1 mL of each enriched media was streaked on the solid media. All bacterial and fungal strains used in this work were selected from previous studies and identified correctly by MALDI-TOF-MS [13–15,21].

Moreover, to determine the sensitivity of our *C. auris*-specific medium, a series of ten-fold dilution (10⁻¹ to 10⁻¹⁰) of 0.5 McFarland of each *C. auris* clade was cultured. For the second clade, we tested the strain of DSMZ collection: DSM 21092. In addition, we also cultured a mixture of the same ten-fold dilutions of 0.5 McFarland of each *C. auris* strain with a stool sample negative in real-time PCR for *C. auris* (10⁻¹ to 10⁻¹⁰). We did this as an attempt to determine whether the natural presence of microbes in stools would affect the growth of *C. auris* on our designated medium.

3. Results

We first cultured all our available *Candida* spp. strains (*n* = 135) on the broth developed by Welsh et al. Interestingly, we isolated 100% of the tested *C. auris* (*n* = 7) and *C. tropicalis* (*n* = 6) species on the Welsh broth [5].

However, when we cultured all strains on a combination of MacConkey agar and the Welsh broth, the growth of *C. tropicalis* strains was totally inhibited. On the other hand, we observed a normal growth of all *C. auris* species.

The addition of crystal violet, with or without bile salts, did not affect the growth of *C. auris* (Figure 1), which we evaluated by measuring the turbidity of the broth using a spectrophotometer. However, we observed a total inhibition of all *C. tropicalis* strains in the presence of 0.5 mg/L crystal violet.
The addition of bile salts only partially inhibited the growth of *C. auris* clade II. This was reflected as a decrease in the spectrophotometer value in comparison to the negative control only containing broth and crystal violet. As for *C. tropicalis* strains, the presence of bile salts without crystal violet did not inhibit their growth. It was then excluded from the final composition of the SCA medium (Table 2).

Therefore, the growth of *C. tropicalis* was inhibited by adding crystal violet at 0.5 mg/L to the initial broth suggested by Welsh et al. with no effect on the growth of the 7 *C. auris* strains (2 strains/clade: except for clade II (one strain)) in solid and liquid phase.

Moreover, the specificity of this medium was also confirmed after cultivating all bacterial spp. *Candida* spp. and fecal samples mentioned in Table 1 on the last composition of our medium (Table 3). No growth was noted after 3 days of incubation at 40 °C.

### Table 3. Final composition of SCA (Specific *Candida auris*) medium.

| Pancreatic Digest of Casein | Peptic Digest of Animal Tissue | NaCl | Mannitol | Crystal Violet | Agar | pH | Chloramphenicol | Gentamicin |
|----------------------------|-------------------------------|------|----------|----------------|------|----|----------------|-----------|
| Welsh et al. broth         | 5 g                           | 5 g  | 100 g    | 20 g           | -    | 5.6| 50 mg/L        | 50 mg/L   |
| SCA medium                 | 5 g                           | 5 g  | 100 g    | 20 g           | 0.5 mg| 15 g| 7              | 50 mg/L   |

Concerning the sensitivity of our medium, after cultivation of several dilutions of a solution of 0.5 McFarland (10^{-1} to 10^{-10}), the limit of detection (LOD) of *C. auris* Clade I, II and VI was 10^2 CFU/mL in both serial dilutions (with physiological water and a fecal sample). For the third clade (South Africa), we observed an excessive growth of both strains at an LOD of 10^1 CFU/mL. Thus, the presence of other microbes in a fecal sample does not inhibit or affect the growth of *C. auris*. All resulting colonies were isolated and subsequently identified by MALDI-TOF-MS and real-time PCR [9].

The final composition of our medium in 1 L of deionized water was 5 g of pancreatic digest of casein, 5 g of peptic digest of animal tissue, 20 g of mannitol, 0.5 mg of crystal violet (Sigma-aldrich, Darmstadt, Germany), 100 g NaCl, 50 mg/L of chloramphenicol and 50 mg/L of gentamicin with pH = 7 (±0.2) at 40 °C (Figure 1, Table 3).

### 4. Discussion

Correct identification and rapid isolation of *C. auris* is essential for the timely and appropriate antifungal treatment and for infection prevention and control measures. This ensures limiting and controlling a possible nosocomial outbreak of a pathogen with fatal consequences and limited therapeutic options. This need is emphasized in the current COVID-19 pandemic we are living in, whereby an increasing amount of critically ill, immunocompromised patients populate the hospitals [22-24]. The gold standard of diagnosis remains molecular identification by sequencing and/or real-time PCR [9-12]. However, this is expensive and may only be available at specialized referral or research laboratories. Therefore, we have developed a cheap, simple, easily-prepared medium for isolating *C. auris* that is specific and sensitive.

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Figure 1. Striking *Candida auris* strains onto SCA medium, 2 strains/clade except for *C. auris* clade II (one strain).
A caveat to the enrichment broth developed by Welsh et al. for *C. auris* isolation is that it also grows *C. tropicalis* strains, which we demonstrated in our work. We show that the addition of crystal violet (0.5 mg/L) to our specific *C. auris* medium inhibited the growth of *C. tropicalis*. SCA (specific *C. auris*) is considered as a new version of the above-mentioned broth [5].

In addition, other selective media are recently described for the isolation of *C. auris* [25–27]. Generally, the selected criteria to cultivate this yeast remain the same as those used in our work: the thermo-resistance, halo-tolerance and multi-resistance to many antifungal agents [2,4]. Here, we managed to develop a medium with the minimum possible inhibitors (40 °C incubation instead of 42, and 10% salinity instead of 12.5% compared to another study [26]). These moderate inhibitors still maintain the selectivity of SCA medium for all *C. auris*.

Usually, *C. auris*, *C. krusei* and *C. parapsilosis* appear as pink colonies on the CHROMagar medium [2,4,28], and a newer version of CHROMagar has been developed to identify *C. auris* [25,27]. This phenotypic identification may be biased and reader-dependent, since it is based on interpretation of the color/morphology of growing colonies. Moreover, a longer duration of incubation may lead to a change in results as well. Thus, a high percentage of error may occur. Our SCA medium facilitates the interpretation of results by only selecting for *C. auris*.

Interestingly, spiking a stool sample with a *C. auris* strain affected neither the specificity nor the sensitivity of this medium. However, increasing our sample size and testing a larger amount of clinical *C. auris* isolates and related yeasts (such as *C. famata*, *C. pseudohaemulonii*, *C. metapsilosis*, *C. orthopsilosis*, *C. rugosa*, *C. vulturna*, *Wickerhamomyces anomalus* and *C. sake*) is necessary for further evaluation, validation and reproducibility of our *C. auris*-specific medium.

5. Conclusions

The use of our developed medium enables the rapid, specific isolation of *C. auris* strains and helps in the timely management of patients and resources to limit the occurrence of *C. auris* outbreaks. We propose an implementation of SCA medium in routine clinical mycology for screening skin, urine, vaginal and blood samples, especially in high-risk populations [13,15]. This SCA medium will further enhance our understanding of the phenotypic characteristics of *C. auris* and future isolates, most importantly allowing an accurate study of their antifungal resistance profiles.

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Institutional Review Board Statement: Ethical review and approval were waived for this study, as this research does not involve human body/tissue, and only discarded stool samples, once laboratory analyses had been initiated, were used. According to French law (Loi no 2012–300 du 5 March 2012 and Décret no 2016–1537 du 16 November 2016 published in the ‘Journal Officiel de la République Française’), no Institutional Review Board is necessary in this case.

Informed Consent Statement: Patient consent is not required for this type of study (Loi no 2012–300 of 5 March 2012 and Décret no 2016–1537 of 16 November 2016 published in the ‘Journal Officiel de la République Française’).

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