

## Use of pea agar with Tween 80 for Dalmau technique for identification of *Candida albicans*

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### ABSTRACT

**Introduction:** *Candida albicans* produces devastating infections with considerable attributable mortality. Identification rests of Dalmau technique using rice extract agar, which is very prone to aerial contamination and drying and breakage. **Materials and Methods:** We here evaluated pea agar, with and without Tween 80, as medium for Dalmau technique for identifying *C. albicans* and compared it with rice extract agar. **Results:** Pea agar with Tween 80 was a very useful medium for identifying *C. albicans*. **Conclusion:** Pea agar with Tween 80 can be used for Dalmau technique for *C. albicans* identification.

**Key words:** *Candida albicans*, Dalmau, Pea agar

*Candida albicans* causes a plethora of infections in man such as oral thrush, candidemia, and urinary infection [1]. Identification depends mainly on microscopic morphology observation by Dalmau technique using rice extract agar or corn meal agar, in which *C. albicans* produces single terminal chlamydo spores at 25°C after 48 h of incubation. Of these, rice extract agar, discovered by developed by Taschdjian, is used often and has proved to be a good medium for this purpose [2]. However it has some disadvantages like it has propensity to get broken, discolored easily and contaminated by airborne microflora [3]. Hence other media should be looked for identification of *C. albicans* by Dalmau technique, since identification is a prerequisite for proper antifungal treatment. Keeping this thing in mind, our study was aimed at evaluation of pea agar, with and without Tween 80, for Dalmau technique for identifying *C. albicans* clinical isolates and comparing it with rice extract agar.

### MATERIALS AND METHODS

This was a lab based observational study carried out in the department of microbiology of the institute from March to September 2016, as part of short time summer training approved by institute ethics committee. In first, 10 different clinical isolates of *C. albicans* were randomly selected from the clinical isolates in the lab recovered from routinely processed samples such as urine, pus, and others. All yeast isolates were identified using germ tube test, sugar fermentation, and Dalmau technique using rice extract agar (composition: Rice 0.04 g, agar 1.5 g, and deionized water 100 ml). They were streaked also on pea agar, with or without

Tween 80, in 3-4 zigzag lines through partial thickness of medium and at room temperature, incubated for 48 h and observed microscopically.

Formula of pea agar was as follows:

- Smashed green pea: 1 g
- Agar: 1.5 g
- Deionized water: 100 ml

Formula of pea agar with Tween 80:

- Smashed green pea: 1 g
- Agar: 1.5 g
- Tween 80 (Polysorbate 80): 100 µl
- Deionized water: 100 ml.

They were sterilized by autoclaving at 121°C for 15 min at 15 lbs/in<sup>2</sup> pressure and poured in sterile polythene Petri dishes.

Isolates were streaked in 3-4 zigzag line by slit inoculation through partial thickness of medium, and covered with flame-sterilized cover slip. Plates were incubated in dark, at room temperature for 48 h and then observed microscopically under ×10 and ×40 objective. Plates were also observed for drying, discoloration or cracking, if any, visually. All these experiments were conducted thrice with each isolate.

### RESULTS

Pea agar with Tween 80 was found to be almost as efficient as compared to rice extract agar for identification of *C. albicans* isolates. Single terminal chlamydo spores were observed in 80% cases using pea agar with Tween 80, as compared to rice extract agar. However, plain pea agar was no found to be as

good (terminal chlamydospore found in only 25% cases as compared to rice extract agar). Pea agar with Tween 80 was also found to be susceptible to less aerial contamination by bacteria and filamentous fungi as compared to rice extract agar. There was also less cracking and discoloration on prolonged incubation at room temperature when compared to rice extract agar.

## DISCUSSION

Rice extract agar has been used successfully since many years for identification of *C. albicans* in the microbiology laboratory, but suffers from certain disadvantages such as frequent aerial contamination and cracking. Green pea is a good nutrition source for humans; it has 8% available carbohydrates, 0.1% fat, and 35% dietary fiber [4]. Hence, it is not very rich in carbohydrates and for fungi, and it can be used as component of nutritionally poor medium for Dalmau technique. As a component of Andersen's pork pea agar, it has been employed for culturing *Clostridium* spp. from clinical samples [5]. To the best of our knowledge, pea agar has not been used for Dalmau technique. This is a good finding and hence, pea agar with Tween 80 can be used as a substitute of rice extract agar for Dalmau technique. *C. albicans* is a smart pathogen causing numerous human infections [6]. Its identification is an important for proper treatment initiation. Identification requires Dalmau technique on corn meal agar or rice extract agar or any other suitable medium, which needs 3 days or more incubation at room temperature and subsequent observation of typical microscopic morphology [7]. A medium which gets contaminated, cracked or discolored easily is hence unsuitable for his purpose. Walker and Huppert, in 1960, reported that the addition of Tween 80 to Corn Meal Agar resulted in very rapid and abundant chlamydospore formation in *C. albicans* isolates [8]. Hence, we tried this detergent in pea agar also and obtained very good results. According to our study, PEA agar with Tween 80 could circumvent some of the disadvantages of rice extract agar, such as airborne contamination and breakage.

Further studies are required in this regard so that routine usage of this medium in diagnostic mycology can be advised.

## CONCLUSION

Pea agar with Tween 80 can be used comfortably for Dalmau technique for *C. albicans* identification from clinical samples.

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