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## Reproducibility of CSF quantitative culture methods for *Cryptococcus neoformans*

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**Background:** Quantitative cryptococcal cultures provide a measure of disease severity in cryptococcal meningitis, and the rate of fungal clearance by quantitative culture has become a widely accepted surrogate outcome measure in phase II clinical trials. Various quantitative methodologies have been used to quantify CSF fungal burden; however, the reproducibility of between techniques is unknown.

**Methods & Materials:** 213 CSF samples were prospectively collected from 70 individuals with cryptococcal meningitis at Mulago Hospital in Kampala, Uganda during Sept-Nov 2013. Each sample was simultaneously cultured by three different quantitative culturing techniques: 1) "standard" 100mcL input volume of CSF with an additional five 1:10 serial dilutions; 2) a AIDS Clinical Trials Group (ACTG) method using various input volumes (1000,100,10mcL) and two 1:100 dilutions with (100,10 mcL input volume) per dilution; 3) 10mcL calibrated plastic loop of undiluted and 1:100 diluted CSF. Colony forming units (CFU)/mL were quantified on the tenth day of culture. In addition, CSF at time of diagnosis was analyzed by automated cell counter and cryptococcal antigen (CRAG) lateral flow assay (LFA) titers.

**Results:** Mean log10 transformed CFU counts suggested no significant differences between the standard method and either of the two alternative methods by paired t-test (difference=+0.036 ACTG,p=.690;-0.053 log10CFU/mL loop, p=.671), although the ACTG and loop methods differed significantly (difference=+0.55,p=.001). Correlation between tests was high at r=0.82, 0.85, and 0.83 for the standard-ACTG, standard-loop, and ACTG-loop methods, respectively. A weighted kappa statistic allowing for 1 log10 difference between methods showed moderate agreement between all tests, with k=0.50, 0.57, and 0.45 for the standard-ACTG, standard-loop, and ACTG-loop methods, respectively. No significant relationships were identified between culture methods and automated cell counts (diff=-1.13,p<0.001 vs. standard, R2=0.49). Regression analysis showed a significant association between being in the highest tertile of LFA titers and higher CFUs by the standard method (p=.042)

**Conclusion:** Overall, the three methods of quantitative culture produced highly comparable but not identical results. There were significant differences between ACTG and loop techniques. The choice of quantitative method should be made based on lab-specific capacity and reproducibility of each technique.

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## Mechanism of inhibition of biofilms in *Candida albicans* by phenyl aldehydes and propanoids

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**Background:** The majority of manifestations of candidiasis are associated with the formation of *Candida* biofilms that are notoriously resistant to antimicrobial agents and withstand host immune defenses making biofilm-associated infections refractory to conventional antibiotic therapy. This has encouraged the researchers to investigate the mechanisms of biofilm development so that newer and effective anti-biofilm agents, targeting structural complexity of biofilms, could be exploited. Therefore, this study was conducted to investigate biofilm formation in 20 azole-resistant strains of *Candida albicans* of clinical origin (vaginitis, urinary tract infections and candidemia). Also, to study the correlation of biofilm formation with the cell surface hydrophobicity and production of biosurfactants in these strains. Furthermore the effects of two phyto-compounds of functional group phenyl aldehydes and propanoids namely cinnamaldehyde and eugenol on the production of biosurfactants by these strains was studied.

**Methods & Materials:** XTT reduction assay, spectrophotometric analysis, light microscopy and scanning electron microscopy (SEM) were employed to determine the effect of test compounds on the *Candida* biofilms. The Ring method using a DuNouy Tensiometer and Liquid Chromatography-Mass Spectrometry (LC-MS) techniques were used to determine the production of biosurfactant.

**Results:** Most of the *C. albicans* strains tested displayed formation of moderate to strong biofilms. Preformed *Candida* biofilms showed ≥1024 times increased tolerance to antifungal drugs. There was good relationship among hydrophobic strains to form moderate to strong biofilms. Seventy percent of the test strains exhibiting hydrophobicity, formed moderate to strong biofilms ($OD_{280}$ 0.5->1.0). Among these, strains *C. albicans* 05, 06, and 18 were strongly producing biosurfactant and reduced the surface tension from 69 to 19.1 mN/m. The LC-MS analysis has revealed production of sophorolipid of molecular weight 615/740 from *C. albicans* 14. Treatment of cells with cinnamaldehyde and eugenol resulted in the decreased production of biosurfactants and, distorted biofilms as observed under SEM.

**Conclusion:** Our study highlights that production of biosurfactants play a role both in maintaining channels between multicellular structures in biofilms and in dispersal of cells from biofilms. Inhibition of production of bioisurfactants by the test compounds indicates that phenyl aldehydes and propanoids or other related compounds could play important role in developing anti-biofilm agents.

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