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Rapid detection of *Cryptococcus gattii* sensu lato using gold nanoparticles

Detección rápida de Cryptococcus gattii *sensu lato mediante nanopartículas de oro*

Dear Editor,

Successful treatment of cryptococcosis requires rapid, accurate identification of *Cryptococcus neoformans* or the *Cryptococcus gattii* species complex in addition to their discrimination from other pathogenic yeast species. Gold nanoparticles (AuNPs) have enabled improvements in biomedical sciences owing to their versatile properties and capacity for detecting biological molecules at low concentrations; these methods are inexpensive, rapid, and stable.[1,2,6–9] We describe the application of AuNPs for detecting *C. gattii* sensu lato.

Our protocol was approved by the Animal Research Ethics Committees (23108.014032/14-0) and Research Ethics Committees of Hospital Universitário Júlio Muller (888/CEP-HUJM/2010). Twenty-one clinical *C. gattii* sensu lato isolates and four *C. neoformans* sensu lato isolates from humans and animals were tested. DNA was extracted using the glass bead method[3] and samples representing each of the eight major molecular types of the *C. neoformans/gattii* species complex were used (Table 1). DNA samples from non-*Cryptococcus* microorganisms served as controls (Table 1).

AuNPs were synthesized using the citrate reduction method.[4,5] Briefly, 250 ml of 1 mM gold chloride was boiled and added to 25 ml of 38.8 mM sodium citrate. For the hybridization test, we used probes based on the superoxide dismutase (*SOD1*) gene of *C. gattii* sensu lato (*SOD1*CGF 5′GATCCTCACGCCATTACG3′ and *SOD1*CGR 5′GAATGATGCGCTTAGTTGGA3′). First, 50 ng of sample DNA was denatured at 95 °C for 3 min in a mixture containing 1.25 M NaCl, 20 mM Tris–HCl, and 20 pmol oligonucleotides, followed by annealing at 50 °C for 2 min for hybridization and holding at 4 °C. This was followed by addition of 50 μl of the prepared AuNPs. Absorption (OD_{300}–OD_{800}) was measured using the BioTek® spectrophotometer (Winooski, VT, USA). The cutoff point was determined using the average absorbance values minus two standard deviations of five control samples without target DNA.

All reference samples were identified as *C. gattii* sensu lato, and a color change from red to purple indicated a positive reaction. Moreover, five non-*Cryptococcus* species and four *C. neoformans*

Table 1
Reference strains representing each of the eight major molecular types of the *Cryptococcus neoformans/gattii* complex, identified using amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP), and non-*Cryptococcus* strains.

Reference strain/isolate	Genotype
C. neoformans sensu lato (WM148)	AFLP1/VNI
C. neoformans sensu lato (WM626)	AFLP1B/VNII
C. neoformans sensu lato (WM628)	AFLP3/VNIII
C. neoformans sensu lato (WM629)	AFLP3/VNIV
C. gattii sensu lato (WM179)	AFLP4/VGI
C. gattii sensu lato (WM178)	AFLP6/VGII
C. gattii sensu lato (WM161)	AFLP5/VGIII
C. gattii sensu lato (WM779)	AFLP7/VGIV
Aspergillus fumigatus (ATCC 204305)	NA[a]
Pichia pastoris (GS115)	NA[a]
Conidiobolus lamprauges[b] (INCQS 40316)	NA[a]
Malassezia pachydermatis[b]	NA[a]
Pythium insidiosum[c] (CBS 101555)	NA[a]

[a] Not available.
[b] Internal transcribed spacer (ITS) sequenced.
[c] Stramenopila.

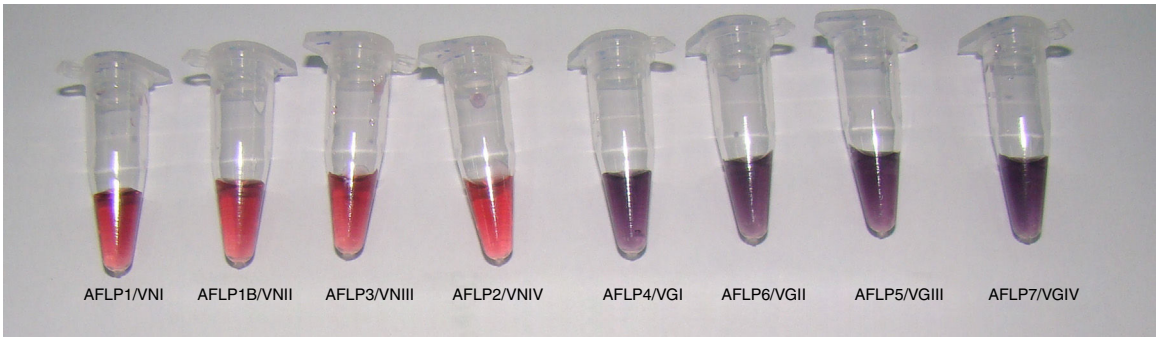

Figure 1. AuNPs test for detecting *C. gattii* based on th *sod* gene. VNI to VNIV: reference strains of *C. neoformans*; VGI to VGIV: reference strains of *C. gattii*. The reddish colour indicates a negative result, the purple one a positive result.

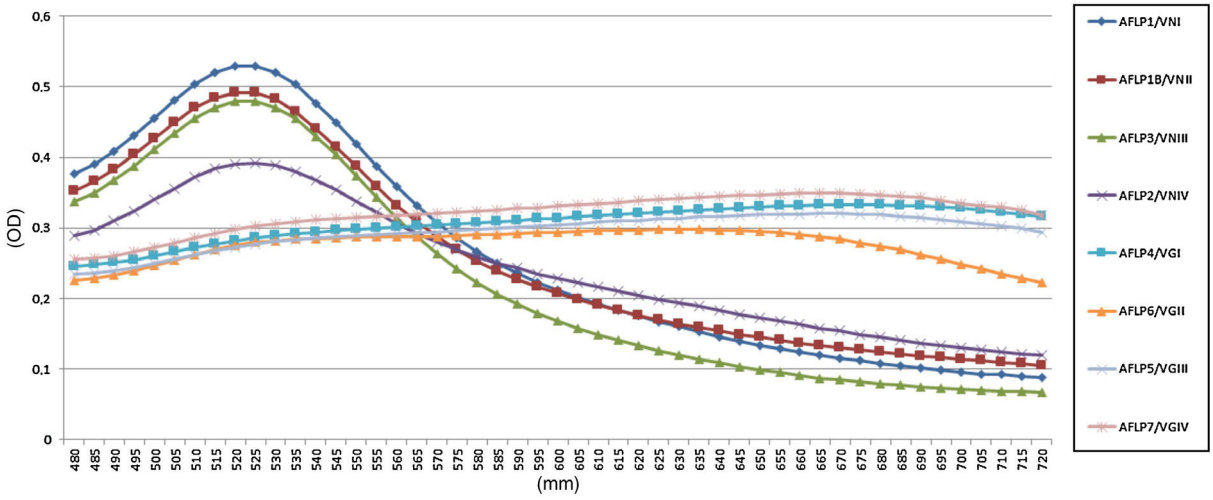

Figure 2. Graphic representation of the scanning absorbance of the standard positive strains of *C. gattii* and the standard negative strains of *C. neoformans*.

sensu lato isolates were subjected to the AuNP assay to evaluate its specificity. All results were negative with no cross-reaction (100% specificity, Fig. 1).

In the AuNP test of the reference *Cryptococcus* strains, there were spectrophotometric signals at the 525 and 655 nm absorbance peaks, indicating negative and positive results, respectively (Fig. 2). The average optical density at 525 nm for the positive reference samples was 0.28 (confidence interval [CI] = 0.27–0.30; SD = 0.009), and the average for the negative samples was 0.47 (CI = 0.39–0.52; SD = 0.05). These results established that a positive sample yields an $OD_{525} \leq 0.37$. Twenty-one (100%) clinical isolates of *C. gattii* sensu lato showed macroscopic positivity and OD_{525} values between 0.24 and 0.30, i.e., below the cutoff value for a negative result.

In summary, AuNPs serve as an alternative tool for developing new methods for clinical diagnosis; our AuNP-based test allowed rapid and specific identification of *C. gattii* sensu lato isolates. Its main advantages are its simplicity, ease of use, and low cost for set up without the need for sophisticated equipment.

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Querion de Celso de la vulva: una enfermedad común en una localización infrecuente

Kerion of the vulva: A common pathology in an unusual location

Las dermatofitosis cutáneas son infecciones muy comunes producidas en la piel por un grupo de hongos denominados dermatofitos, conformado por los géneros *Trichophyton, Microsporum* y *Epidermophyton*, que afectan a las capas superficiales queratinizadas de la epidermis y anejos cutáneos[8]. Sin embargo, existen dermatofitosis denominadas profundas como el querion de Celso, cuya localización más frecuente es el cuero cabelludo y se manifiesta clínicamente como una erupción pustulosa grave con intensa reacción inflamatoria y exudación purulenta[8]. A continuación describimos una localización atípica o poco frecuente de esta enfermedad.

Una mujer de 19 años de edad fue remitida a dermatología desde el servicio de ginecología por presentar lesiones pustulosas en la zona del pubis. La paciente había acudido en varias ocasiones a urgencias por el dolor local, donde se le había prescrito tratamiento antibiótico con amoxicilina-ácido clavulánico 875-125 mg/8 h/7 días y analgesia oral e intravenosa.