

# Rapid diagnosis of vaginal candidosis by latex particle agglutination

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**SUMMARY** Vaginal swabs from women who on clinical evidence were thought to have vaginal candidosis were examined for yeasts by conventional laboratory methods (microscopy and culture) and also assayed for *Candida* antigens using a rapid (3 min) slide latex particle agglutination test. Results showed that a diagnosis of vaginal candidosis based on clinical criteria alone is unreliable: only half of the women were subsequently confirmed as having candidosis by microscopy and culture. The new slide latex particle agglutination test gave better results, with 100% specificity, 80% sensitivity, high predictive values ( $\geq 91\%$ ), and an overall diagnostic efficiency of 93%.

From the results of this preliminary study, slide latex particle agglutination looks a promising, rapid alternative to conventional laboratory methods for confirming a clinical diagnosis of vaginal candidosis and has the considerable advantage that it can be conveniently used in a clinical setting.

*Candida* vaginitis (thrush) is one of the most frequently encountered forms of superficial candidosis. It usually arises due to alterations in the normal physiological state of the host and is a common infection in, for example, poorly controlled diabetics,<sup>1,2</sup> women taking oral contraceptives,<sup>3-5</sup> and in those in the third trimester of pregnancy.<sup>6,7</sup> Vaginal candidosis presents mainly in general practice, but it is also frequently encountered in family planning, obstetrics, gynaecology, and venereology clinics.

A presumptive diagnosis of *Candida* vaginitis may be based on clinical features such as a pruritis, milky white discharge, erythema and oedema, and the presence of white, curd like patches on the epithelial surface of the vulva, vagina, and cervix. Some of these features, however, are also seen in other forms of vaginitis, particularly in infection due to *Trichomonas* or gonorrhoea, and therefore an unequivocal diagnosis of candidosis also requires the demonstration of yeasts in material from lesions by both microscopy and culture.<sup>8</sup> In practice, the diag-

nosis of most cases of vaginal candidosis is made on purely clinical grounds because it is often impracticable or too time consuming to perform the necessary confirmatory tests in a clinical environment and laboratory facilities are not readily available. Moreover, if material is sent to the laboratory there is an inevitable delay before results are available. This less than satisfactory situation means that many women with vaginitis continue to suffer discomfort because of a delay in initiating appropriate treatment and, furthermore, it is costly in terms of the inappropriate medicaments given to some patients.

We have investigated the possibility of rapid (3 min) diagnosis of vaginal candidosis using a slide latex particle agglutination (SLA) test for the detection of *Candida* antigens in vaginal secretions and have compared its efficacy with clinical and conventional laboratory diagnosis.

## Material and methods

An SLA reagent for the detection of *Candida* cell wall antigens was developed and evaluated for the rapid diagnosis of vaginal candidosis. Vaginal swabs from women considered clinically to have *Candida* vaginitis were first examined for yeasts by microscopy and culture and then tested blind with the

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SLA reagent. Results were compared.

#### CLINICAL SPECIMENS

A total of 87 vaginal swabs received by the Regional Mycology Laboratory at the General Infirmary, Leeds, over a period of four months were included in the study. All the swabs had been taken by general practitioners from women considered clinically to have vaginal candidosis and who were taking part in a multicentre trial of a new antifungal treatment. The swabs were sent in transport medium (Sterilin Ltd, Teddington, England) to Leeds for confirmatory laboratory diagnosis. Thirty six swabs were taken from patients before the start of antifungal treatment and the remainder (51) were follow up swabs to assess the success of treatment.

#### LABORATORY DIAGNOSIS

##### *Microscopy and culture*

Material from the swabs was examined microscopically in potassium hydroxide (10% wt/vol) for the presence of yeast cells and mycelium or pseudomycelium. Material was also cultured on Sabouraud's dextrose agar containing chloramphenicol (0.05 g/l) in petri dishes at 37°C and cultures examined at 24 and 48 h. Any yeasts that developed were identified using the germ tube test<sup>9</sup> and the API 20C Aux yeast identification system (API Lab Products, Basingstoke, England).

Women were considered to have *Candida* infection when swabs were positive for yeasts by both microscopy and culture. When microscopy was negative and only small numbers of yeasts (<10 colonies) were recovered in culture, this was taken to indicate colonisation with *Candida* rather than infection.

##### *SLA test*

1 *Preparation of reagents.* An antiserum was raised in New Zealand White rabbits using a partially purified cell wall fraction<sup>10</sup> of *C albicans* 3153, serotype A (ATCC No 28367) as the immunogen. Purified immunoglobulins were obtained from this antiserum by caprylic acid precipitation,<sup>11</sup> followed by diethylaminoethyl Sephadex chromatography. Immunoglobulins were lyophilised and stored under vacuum in sealed ampoules.

Extensive preliminary work was performed to determine the optimum concentration of immunoglobulins required to sensitise the latex particles so as to give maximum sensitivity compatible with stability.

Polystyrene latex particles 0.8 µm diameter (Merrel-Dow Ltd, Hounslow, England) were adjusted to a 1% (vol/vol) suspension in distilled water. Ten millilitres of immunoglobulin, at a con-

centration of 100 µg/ml in glycine buffered saline, pH 8.2 (GBS), was added to 10 ml of latex suspension and the two were mixed vigorously for 2-3 min. Ten millilitres of GBS containing 1% bovine serum albumin (GBS-BSA) was added and mixed thoroughly. The latex reagent was sonicated (12 µm amplitude) for 6 s to break up any aggregates and centrifuged (7000 g for 10 min). After washing twice with GBS-BSA (30 ml) the latex particles were resuspended in 10 ml of GBS-BSA and stored at 4°C.

The sensitivity of the latex reagent for antigen detection was determined by reacting against *C albicans* cell wall mannan<sup>12</sup> diluted in GBS, and its reactivity was checked every month to ensure that it had not deteriorated. The reagent used for the study could detect 500 ng/ml mannan in GBS.

2 *Test procedure.* Swabs were agitated in 100 µl of GBS for 1-2 min and the washings centrifuged (1500 g for 5 min) to remove particles of transport medium. Twenty five microlitres of sensitised latex reagent were added to 25 µl of supernatant on a black glass slide and the two reagents were mixed thoroughly with an applicator stick. After manual rotation of the slide for 3 min, agglutination was recorded macroscopically on a scale of negative to 4+ (coarse agglutination, background clear). Swabs giving an agglutination score of 1+ (fine granular agglutination, background milky) or greater were recorded as positive.

#### Results

##### CLINICAL AND LABORATORY DIAGNOSIS

Of the 36 vaginal swabs taken from women with clinically suspected vaginal candidosis before treatment, 18 (50%) were subsequently confirmed as

*Evaluation of slide latex particle agglutination (SLA) test for the diagnosis of vaginal candidosis. Comparison of the number of patients with suspected infection who were positive by microscopy and culture and by SLA.*

	Latex test		
	Positive	Negative	Total
Vaginal candidosis*			
Positive	24 (a)	6 (b)	30 (c)
Negative	0 (d)	57 (e)	57 (f)
Total	24 (g)	63 (h)	87 (i)

\* Vaginal candidosis positive = microscopy and culture positive.  
 Vaginal candidosis negative = microscopy positive and culture negative;  
 microscopy negative and <10 yeast colonies on culture.

Sensitivity (a/c) = 80%; specificity (e/f) = 100%; predictive value positive (a/g) = 100%; predictive value negative (e/h) = 91%; efficiency ( $\frac{a+e}{i}$ ) = 93%

cases of *Candida* infection by conventional laboratory investigations (microscopy and culture). Most of the infections (15) were caused by *C albicans*, although in two instances *C parapsilosis* was responsible and in one case *C glabrata*.

#### SLA TEST

The results obtained with the latex reagent are summarised in the Table. Of the 87 vaginal swabs tested, 30 (34%) were positive by microscopy and culture, and of these 24 (80%) were also positive with the SLA reagent. False negative results were obtained in six (20%) instances, but no false positive results were obtained.

#### SLA PERFORMANCE

The latex reagent had an excellent specificity (100%) and consequently a predictive value positive of 100%. The sensitivity was 80%, giving a predictive value negative of 91% and an overall efficiency of 93%.

#### Discussion

*Candida* could not be recovered from half of the women who were clinically thought to have vaginal candidosis. It is unlikely that the failure to detect yeasts in swabs from these women can be attributed to the poor sensitivity of the tests employed; in most of those women found to be positive by the laboratory, numerous yeast cells and mycelium could be seen on direct microscopy and large quantities of *Candida* were recovered in culture without any difficulty. Rather, the results confirm the difficulty of diagnosing vaginal candidosis on purely clinical grounds and the desirability of carrying out confirmatory laboratory tests. The lack of facilities for such tests, the expertise required, and the time delay before results are available make microscopy and culture less than satisfactory. Clearly, however, any new test for the diagnosis of these infections would need to be rapid and easy to perform to confer any advantage over these conventional laboratory procedures. An SLA test with the required sensitivity and specificity would fit these requirements.

Latex particle agglutination tests were first described for the diagnosis of rheumatoid arthritis.<sup>13</sup> Since then, the production of new latex reagents has enabled the development of rapid and more sensitive agglutination assays, which have gained wide application for the diagnosis of bacterial infections.<sup>14-16</sup> In mycology, a latex test for the detection of antigen has been used routinely for the diagnosis of cryptococcosis since 1963,<sup>17</sup> and more recently an SLA test has been described for the detection of

antigenaemia in patients with systemic candidosis.<sup>18</sup>

The SLA reagent evaluated in this study had total specificity with high predictive values, and even though there is scope for improving its sensitivity (80%), it was still considerably better for the diagnosis of vaginal candidosis than reliance on clinical features alone.

The false negative latex results seen in this survey did not appear to be related to the quantity of *Candida* grown from the swabs since most swabs gave profuse (confluent) growth of yeast on culture, and, conversely, swabs giving fewer colonies on culture scored up to 3+ by SLA. There are a number of other possible explanations. False negative reactions could, for example, be obtained in infections caused by *Candida* species other than *C albicans*. In this survey one of the false negatives in fact occurred with the *C glabrata* infection, although the two *C parapsilosis* infections both gave positive results in the SLA test. Furthermore, it is possible that some false negative reactions were obtained with infections caused by isolates of the less frequently encountered *C albicans* serotype B. The antibodies used to sensitise the latex particles in this study were raised against *C albicans* serotype A and differences in cross reactivities between the cell wall antigens of the two serotypes, as well as between different *Candida* species, have been shown.<sup>19</sup> It is most likely, however, that the majority of the false negative reactions in this survey resulted from the removal of a substantial proportion of the material from the swabs for microscopy and culture before testing with the SLA reagent. The priority given to conventional laboratory methods was dictated by the fact that swabs were primarily collected for assessment of patients in an antifungal trial. Swabs taken from patients and tested directly by SLA would not suffer this disadvantage and, moreover, would not require the centrifugation step used in this survey to remove particles of transport medium. The use of latex particles coated with antibodies to a wider range of *Candida* species/serotypes may nevertheless contribute to an improved sensitivity and this aspect is under investigation. Even as it stands, the test gives acceptable results and is worthy of further evaluation.

In conclusion, this preliminary study shows the feasibility and potential of SLA for the rapid confirmatory diagnosis of vaginal and possibly other forms of superficial candidosis. The test can easily be carried out in a clinical setting and so offers considerable benefits to doctor and patient.

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