Apparatus and method for immediate diagnosis of exudative vaginal yeast infections, using a sample of vaginal discharge, dyeing the yeast present in the discharge with a fluorescent dye specifically sensitive to yeast, and subjecting the dye to ultraviolet radiation to determine the level of visible fluorescence, with the level of fluorescence indicating the presence or absence of the high number of yeast associated with a vaginal infection.
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Specification

APPARATUS AND METHOD FOR IMMEDIATE DIAGNOSIS
OF VAGINAL YEAST INFECTIONS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to a method
and apparatus for immediate diagnosis of exudative vaginal
yeast infections. In particular, this invention relates
to a method and apparatus which permit diagnosis or
ruling-out of vaginal yeast infections without culturing
yeast, thereby permitting immediate diagnosis through
fluorescence of a dyed sample of the vaginal exudate.

Brief Description of the Prior Art

Vaginal yeast infections are a common problem
affecting women of all ages. Vaginal anti-yeast
prescriptions numbered 13 million in 1990 in the U.S. The
infection results from the overgrowth of yeast which are
often normally present but in much smaller numbers. The
symptoms of a yeast infection include vaginal itching,
discharge, soreness, irritation or burning. Since a
vaginal yeast infection is strictly due to the presence of
large numbers of the responsible organism, diagnosis and
treatment would seem to be simple. However, other more
serious vaginal infections can present a similar clinical
picture which usually includes local itching, a vaginal
discharge, and possibly abdominal pain and fever. For
example, bacterial vaginosis, bacterial trichomoniasis,
chlamydial infections, and gonorrhea can resemble yeast
infections, especially to women without medical training.

Presently, over-the-counter anti-yeast medications
have been approved for treating vaginal yeast infections.
The availability of these medications encourages women to
self-diagnose and self-treat a potentially serious medical
problem, without a medical examination, based upon a hope
that the problem may simply be due to yeast. Self-
diagnosis without diagnostic data is dangerous, since
proper treatment may be delayed or the wrong treatment may be undertaken, possibly leading to invasive infections or sterility due to pelvic inflammatory disease.

The prior art teaches confirmation of the presence of an infectious organism through the use of a variety of culture techniques. U.S. Patents Nos. 3,368,569 and 4,953,560 teach use of a swab including a culture medium. U.S. Patents No. 3,616,265, 4,653,510, and 4,485,824 teach a variety of swabs and culture mediums for simplification of transferring a vaginal secretion to a culture medium.

All of these techniques require that the yeast be cultured, a technique that requires incubation of the yeast for 12 to 24 hours under aseptic conditions with complex agar media by medical personnel. Therefore, none of these devices provide an immediate method for diagnosis of the presence of the high number of yeast associated with a vaginal yeast infection, and none of them are applicable to a test which may be used at home by persons who are not medically trained.

SUMMARY OF THE INVENTION

It is a primary object of the present invention to provide a method and an apparatus for immediate and inexpensive determination of whether the large numbers of yeast associated with vaginal yeast infections are present.

It is a further object of the present invention to provide a method and an apparatus which are easy to use so that a woman may immediately determine whether or not a high number of vaginal yeast are present, indicating a possible yeast infection, or alternatively whether symptoms associated with a yeast infection may be due to some other more serious problem with similar symptoms.

Another object of the present invention is to provide a simple, self-administered, inexpensive, accurate and reliable test system that allows a woman to immediately determine if she has an overgrowth of vaginal yeast to insure that diagnostic data is available before treatment is begun.
A further object of the present invention is to provide a test that indicates whether treatment for yeast infection is inappropriate, thereby saving critical time and indicating that a medical visit is necessary instead. Briefly, the preferred embodiment of the present invention is a method and apparatus for immediately detecting the presence of high numbers of vaginal yeast which are associated with yeast infections through a method and apparatus which use a sample of vaginal discharge, dyes the yeast present in the discharge with a fluorescent dye specifically sensitive to yeast, and subjects the dye to ultraviolet radiation to determine the level of visible fluorescence. The level of fluorescence indicates the presence or absence of the high number of yeast associated with a vaginal infection.

The attainment of the foregoing and related objects, advantages and features of the invention should be more readily apparent to those skilled in the art after review of the following more detailed description of the invention.

IN THE DRAWING

Fig. 1 is a plan view of the preferred embodiment of a kit for immediate diagnosis of vaginal yeast infections in accordance with this invention; and Fig. 2 is a perspective view of a specimen slide designed for use with this invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

This invention constitutes a method and an apparatus for immediate diagnosis of vaginal yeast infections. With reference to Fig. 1, a yeast detection test kit 10 is shown. Kit 10 includes a kit body with lower test kit body 12 and upper test kit body 14. The test kit body is preferably formed of an inexpensive but durable material, such as plastic. For reasons discussed below concerning the fluorescence method of detection used, the test kit body is preferably black to absorb any stray light and to make the detection method more sensitive by making
fluorescence easier to perceive. The test kit body
includes a closing tab 16 which attaches a locking piece
(not shown) on lower test kit body 12.

Lower test kit body 12 contains the elements of the
test kit used in conducting the yeast infection test.
These include a fluorescence light housing 18 which
includes a fluorescence light bulb 20, on/off switches 22,
and a battery (not shown) to supply power to the
fluorescence light bulb 20. The preferred embodiment uses
near-ultraviolet light to diagnose yeast infections
through visible fluorescence of a dyed yeast sample.
Therefore, a battery-powered near-UV ultraviolet light is
used. An example of a suitable battery-powered
fluorescent lamp which has proved suitable is Radio Shack
Catalog No. 61-2734, using a black light UV bulb No.
F4T5BLB made by WKO, in Japan.

Lower test body 12 also includes a snap-in holder 38
which is used for accurately positioning a specimen slide
40 relative to fluorescence light bulb 20.

Upper test kit body 14 includes the materials
necessary for obtaining and preparing a vaginal exudate
specimen for testing. Test equipment compartment 20 holds
specimen slides 26, cover slips 25, and swabs 24. Cover
slips 25 are preferably glass, and swabs 24 are preferably
composed of cotton or some other absorbent material. A
more detailed view of slides 26 is shown in lower test kit
body 12 as slide 40 and in Fig. 2. Specimen slide 40 has
an area for a control portion 42 and an area for an
exudate portion 44. Preparation of the specimen slide 40
is described in further detail below.

With reference to Fig. 2, an embodiment of a specimen
slide 40 is shown in perspective view. A glass or black
plastic slide is the present preferred embodiment, but an
absorptive slide as shown in Fig. 2 may be used. The
absorptive slide includes a top layer 52 which is
comprised of a 1 micron meter milipore non-cellulose filter
paper, which is dyed black so that fluorescence from the
slide is easier to detect. The middle layer 54 is
comprised of absorptive filter paper, and the bottom layer
56 is comprised of a rigid fiberboard support.

Referring again to Fig. 1, upper test kit body 14
also includes a solution compartment 30 which holds three
bottles of test solution: an alkaline treatment solution
32, a dye solution 34, and a rinse solution 36.

Yeast infection test kit 10 is designed to facilitate
simple and immediate diagnosis of yeast infections. The
kit allows a woman to perform a test in a quick, simple
and private manner.

The following criteria have been established for
vaginal yeast infections. Vaginal yeast at levels of less
than $10^3$ colony-forming units/milliliter of secretion are
normal vaginal flora, not representing yeast infection and
are present in up to 50% of the female post-puberty
population. Only levels of yeast of $10^4$ colony-forming
units/milliliter of secretion or higher constitute the
overgrowth of yeast diagnosed as a yeast infection.

Therefore, vaginal yeast infection is a relative
concentration diagnosis. It takes approximately $10^3$ total
yeast/milliliter as determined by direct microscopic
hemocytometer count to give one colony-forming unit on a
culture plate. Therefore, yeast infection is only present
when the total concentration of all yeast cells exceeds
approximately $10^7$ yeast/milliliter. Recent studies have
measured infection concentrations exceeding $10^9$ colony-
forming units/millimeter of exudate.

Specimen slides 26 are packaged in a protective
packet, such as plastic or foil, which is preferably
airtight and purged with nitrogen to insure a non-
contaminated and non-oxidizing environment for the
specimen slide 26. The presence of a non-contaminated,
non-oxidizing environment is important because specimen
slide 26 contains a control portion, as shown in the
detail of specimen slide 40 in Figs. 1 and 2. Control
portion 42 of specimen slide 40 contains a concentration
of yeast which correlates with the minimum concentration
found in vaginal yeast infections. As noted above, a
yeast infection is only present when the total
concentration of yeast cells exceeds approximately $10^7$ yeast/milliliter. In general, higher numbers of yeast correlate with more severe clinical symptoms, i.e. higher levels of yeast cause a more severe infection. Therefore, control portion 42 holds a standardized yeast sample of approximately $10^7$ yeast/milliliter. The yeast need not be alive. We have found that a control specimen has sufficient lifetime to permit use of a pre-packaged control over an extended time period (exceeding several months), which may be lengthened by use of packaging to retard contamination and aging of the control specimen. Slide 40 preferably includes a notation (on the slide itself or on its packaging) of the expiration date for the control portion 42 of the slide.

This test method measures total yeast concentration by staining the yeast with a dye, Calcofluor White, which forms a specific chemical bond to cellulose and chitin in the yeast cell wall. Other biological agents present in vaginal discharges are dissolved in the preparation of the specimen slide. When yeast stained with calcofluor white are exposed to ultraviolet light, a green fluorescence is emitted. At the level of $10^7$ total yeast/millimeter (the concentration of the preferred standardized sample), the fluorescence of the stained sample is easily visible to the naked eye. Higher concentrations, as would occur in more severe yeast infections, are brighter.

The test for yeast infection is conducted as follows. First, a slide 26 is removed from its package and placed on paper towels. A sample of vaginal discharge is obtained with a swab 24, and then the swab is dabbed or rolled over the exudate sample portion 44 of slide 40. This is preferably done 1 or 2 times with the slide, at two-minute intervals. Exudate portion 44 is allowed to dry for approximately four or five minutes.

The exudate portion 44 and control portion 42 of specimen slide 40 are prepared by first applying several drops of an alkaline treatment and wash solution 32 to exudate portion 44 and control portion 42. The alkaline treatment solution preferably consists of approximately
10% by weight potassium hydroxide in water, or a similar
alkaline solution such as 10% sodium hydroxide in water.
The potassium hydroxide solution dissolves only non-yeast
structures. The potassium hydroxide solution is allowed
to sit on the sample areas for about thirty seconds. The
slide is then tipped on its side to allow any excess
solution to run off onto the paper towel.

Next, several drops of dye solution 34, preferably
calcofluor white, is added to exudate portion 44 and
control portion 42 of specimen slide 40 and allowed to sit
for about 30 seconds. Calcofluor white, an optical
brightener, is a colorless dye used as a whitening agent
in the textile and paper industry. Because it binds to
cellulose, chitin, and fungal elements, and fluoresces
when exposed to long wavelength UV and short wavelength
visible light, it has been used to demonstrate cellulose
in microorganisms, stain the cell walls of plants, and to
screen specimens for fungal elements. The preferred dye
solution utilizes 0.1 gram calcofluor white M2R (Poly-
sciences, Inc., Warrington, PA, or Sigma Chemical Co., St.
Louis, MO) and 0.05 gram Evans Blue (Sigma Chemical)
dissolved in 100 ml distilled water.

After the dye solution has been applied, the slide is
again tipped to allow excess solution to run off onto the
paper towel. Now, several drops of rinse solution 36,
preferably approximately 10% KOH in water (which does not
affect calcofluor's binding to yeast), are gently placed
on the slide sample areas and allowed to stand flat and
still for about thirty seconds. The slide is then tipped
on its side again to allow any excess solution to run off
onto the paper towel. This further removes any dye not
bound to yeast. A cover slip 25 is placed directly on top
of the sample portions of specimen slide 40 and allowed to
sit for about 5 seconds. The specimen slide 40 is then
turned over and gently pressed down on the paper towel to
express any excess solution.

Slide 40 is turned right side up and placed into
snap-in holder 38, which positions the slide exactly with
respect to fluorescence light bulb 20.
Once specimen slide 40 has been prepared, the kit is taken into a darkened room, and the fluorescence light bulb 20 is turned on. The room is preferably as dark as possible so that the eye-sensitivity in viewing the fluorescence of the specimen slide 40 will be as sensitive as possible. Any bluish-green glow coming from the exudate portion 44 of specimen slide 40 is compared visually to the glow coming from the control portion 42 of the specimen slide 40. When the exudate portion 44 and control portion 42 of specimen slide 40 are compared for relative fluorescence, a fluorescence in the exudate portion 44 greater than or equal to the fluorescence of the control portion 42 of the specimen slide indicates the presence of a concentration of yeast which indicates a yeast infection. If the exudate glow is less bright than the glow from the standard sample area, then a diagnosis of vaginal yeast cannot be made with certainty, and the woman should consult a doctor as soon as possible to determine whether or not a more serious infection is present.

The present invention uses both positive and negative results to obtain useful information concerning the possible causes of the vaginal discharge or discomfort. In particular, if a yeast infection is diagnosed, over-the-counter anti-yeast medication can be used. If the test is negative, the woman has been able to eliminate a yeast infection as the cause for a problem and will not be tempted to self-treat for a yeast infection inappropriately, and will be on notice that a more thorough medical test is required.

The test kit 10 of this invention may be used for multiple diagnosis provided that additional specimen treatment solutions 32, 34 and 36, specimen slides 26, swabs 24, and cover slips 25 are provided as required.

We have compared the results obtained with the method and kit of this invention with the results obtained in a medical clinic, which utilize microscopic evaluation of slides to determine the presence of yeast or other agents. In approximately 98% of the cases, we find agreement
between our results in diagnosing yeast infection and the
clinical microscopic test results.

This invention has been described in terms of a
specific dye, calcofluor white, which stains yeast to
fluoresce blue-green under ultraviolet light. This system
is particularly useful because the excitation light (near
UV and short wavelength visible (purple)) is easily
distinguished from the fluorescence (blue-green) with the
naked eye. However, any dye fluorescence test which
correlates specifically with yeast concentration will work
suitably well in this method. For example, yeast
concentration could be correlated with fluorescence from
fluorescein-labeled anti-yeast antibodies in an immuno-
fluorescence microscopic technique.

This invention has been described in terms of finding
concentrations of yeast in vaginal exudates. However, it
is equally applicable to finding concentrations of yeast
in any fluids or semi-solid matter. The test would be
performed in the matter described above for the
fluid/semi-solid matter of interest.

For example, if urine were used in the sample slide
portion instead of vaginal exudate, the results would be
indicative of a bladder yeast infection. The fluorescent
intensity for such infections would be similar to those
associated with vaginal yeast infections.

Another example is oral yeast infections (thrush).
If the white, semi-solid curd-like exudate of thrush were
used in the sample area, the kit and method described
above would easily show very bright fluorescence in this
essentially solid yeast culture. Use of the kit and
method would be identical, with Q-tip application of the
sample material adjacent to the control sample. The
concentration and amount of yeast in the control portion
would be the controlled variable for various diagnostic
conclusions.

Thus, the amount of yeast in the control portion
could be used for semi-quantitative comparison and
measurement of the yeast levels in any fluid or semi-
solid, whether or not the material is biological or
infection exudate, where one wants a semi-quantitative
measure of yeast amount or concentration. Adjustment of
the yeast concentration in the control specimen allows
calibration of the method.

The visibility of fluorescence to the naked eye
starts at a yeast concentration of one million yeast per
milliliter. For use with the naked eye, the control
portion concentration useful for comparison to samples
must be above this one million level. Below this level
concentrations cannot be determined by the naked eye.
Fortuitously, vaginal and bladder yeast infections begin
at this level. Moreover, in samples of solid exudate, as
in thrush and some vaginal yeast infections, the exudates
are essentially of infinite yeast concentration since they
consist of basically solid yeast culture.

If an electro-optical fluorescent reader and
comparator is employed, yeast concentrations below the one
million yeast per milliliter level can be detected, and
quantitative measurement of both higher and lower levels
is straightforward with use of calibrated control
specimens.

A restriction that applies is that the sample
material must be free of "interfering substances". These
would consist of any plant-derived materials such as
paper, cotton, cork or cellulose-related products. With
regard to the use of Calcofluor White dye, this dye
(Calcofluor White) stains the cellulose and chitin of
fungal cell walls. Therefore, any non-fungal animal or
plant product containing these chemicals would be an
interfering substance.

In body exudates, such as vaginal exudates, urine, or
thrust plaques, the only living source of this cellulose-
chitin material is yeast. In this way, interfering
substances are ruled out by the source of the sample as
well as by the pre-treatment and final rinse of the
materials with 10% KOH. Interfering non-living
substances, such as cotton fibers from clothes or Q-tips,
would either not be present or occur in amounts too low to
be visibly fluorescent to the naked eye. Interfering
substances can occur in feces due to ingestion. In the
absence of large amounts of interfering substances, the
kit and method described above may be used to detect the
amount of yeast present in any fluid/semi-solid material.

Although the present invention has been described in
terms of a specific embodiment, it is anticipated that
alterations and modifications thereof will no doubt become
apparent to those skilled in the art. It is therefore
intended that the following claims be interpreted as
covering all such alterations and modifications as fall
within the true spirit and scope of the invention.

What is claimed is:
1. A test kit for detecting the presence or absence of levels of yeast associated with vaginal yeast infections comprising:
   (a) a test kit body;
   (b) a specimen slide to which a sample of vaginal exudate may be applied;
   (c) a dye for staining yeast in said sample of vaginal exudate, said dye capable of fluorescing in the presence of a light source;
   (d) a light source mounted within said test kit body for exciting fluorescence in said dye;
   (e) a control specimen which when exposed to said light source has a level of fluorescence corresponding to the fluorescence from a known concentration of yeast; and
   (f) means for positioning said specimen slide and said control specimen with respect to said light source, whereby fluorescence may be detected from a dye-stained sample of vaginal exudate and compared with fluorescence from said control specimen, to determine whether said sample of vaginal exudate contains levels of yeast associated with vaginal yeast infections.

2. The test kit of claim 1, wherein the dye is calcofluor white.

3. The test kit of claim 1, further comprising an alkaline solution for treating the sample of vaginal exudate prior to staining the sample with said dye.

4. The test kit of claim 3, wherein the dye is calcofluor white.

5. The test kit of claim 1, wherein the control specimen holds a standardized yeast sample for comparison with said sample of vaginal exudate.
6. The test kit of claim 5, further comprising an alkaline solution for treating the sample of vaginal exudate prior to staining the sample with said dye.

7. The test kit of claim 5, wherein the dye is calcofluor white.

8. The test kit of claim 7, further comprising an alkaline solution for treating the sample of vaginal exudate prior to staining the sample with said dye.

9. The test kit of claim 8, further comprising a swab for obtaining said sample of vaginal exudate and applying said sample to said specimen slide.

10. The test kit of claim 9, wherein the test kit body is black.

11. A method for immediate detection of the presence or absence of levels of yeast associated with vaginal yeast infections comprising:
   (a) obtaining a sample of vaginal exudate;
   (b) staining yeast in said sample with a fluorescent dye;
   (c) exposing said stained sample to a light source capable of exciting fluorescence in said dye; and
   (d) comparing the fluorescence of said stained sample with the level of fluorescence from a control specimen having a level of fluorescence corresponding to the fluorescence from a known concentration of yeast.

12. The method of claim 11, wherein the fluorescence is visible light of sufficient intensity for detection with the naked eye.

13. The method of claim 11, wherein said dye is calcofluor white.
14. The method of claim 11, further comprising the step of treating the sample with an alkaline solution prior to staining the sample with said dye.

15. The method of claim 11, wherein the control specimen contains a known concentration of yeast, and the yeast in said control specimen are stained with said dye at approximately the sample time as yeast in said sample are stained.

16. The method of claim 15, wherein said dye is calcofluor white.

17. The method of claim 15, wherein the fluorescence is visible light of sufficient intensity for detection with the naked eye.

18. The method of claim 15, further comprising the step of treating the sample and control specimen with an alkaline solution prior to staining the sample and control specimen with said dye.

19. The method of claim 18, wherein said dye is calcofluor white.

20. The method of claim 18, wherein the fluorescence is visible light of sufficient intensity for detection with the naked eye.

21. A test kit for detecting the presence or absence of yeast in a fluid at a predetermined concentration level comprising:

(a) a test kit body;

(b) a specimen slide to which a sample of fluid may be applied;

(c) a dye for staining yeast in said sample of fluid, said dye capable of fluorescing in the presence of a light source;
(d) a light source mounted within said test kit body for exciting fluorescence in said dye;
(e) a control specimen which when exposed to said light source has a level of fluorescence corresponding to the fluorescence from a known concentration of yeast; and
(f) means for positioning said specimen slide and said control specimen with respect to said light source, whereby fluorescence may be detected from a dye-stained sample of fluid and compared with fluorescence from said control specimen, to determine whether said sample of fluid contains levels of yeast greater or smaller than the concentration of yeast in the control specimen.

22. A method for immediate detection of the presence or absence of yeast in a fluid at a predetermined concentration level comprising:
(a) obtaining a sample of fluid to be tested for yeast concentration;
(b) staining yeast in said sample with a fluorescent dye;
(c) exposing said stained sample to a light source capable of exciting fluorescence in said dye; and
(d) comparing the fluorescence of said stained sample with the level of fluorescence from a control specimen having a level of fluorescence corresponding to the fluorescence from a known concentration of yeast.