AutoGenomics

INFINITI® Candida Vaginitis QUAD Assay

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

Manufactured by AutoGenomics, Inc., 1600 Faraday Avenue, Carlsbad, CA USA 92008

Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
Schiffgraben 41, 30175 Hannover, Germany

Doc EM-34049E (English) Rev. F(CO 3922) February 2017
Tel: 760-477-2248 www.autogenomics.com

INTENDED USE

The INFINITI Candida Vaginitis (CV) QUAD Assay is an *in vitro* diagnostic test for the detection and identification of five pathogens in genomic deoxyribonucleic acid (DNA) obtained from human endocervical and cervical specimens. The INFINITI CV QUAD is designed to identify the presence of the following five fungal pathogens: *Candida albicans* (CA), *Candida glabrata* (CG), *Candida krusei* (CK), *Candida parapsilosis* (CP), *and Candida tropicalis* (CT). The INFINITI CV QUAD is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

BACKGROUND INFORMATION

Vulvovaginal candidiasis (Candidal vulvovaginitis) is the second most common cause of vaginitis in the United States and the most common cause in Europe.1 An estimated 75 percent of women have vulvovaginal candidiasis at some time in life, and approximately 5 percent of women have recurrent episodes.2, 3-5 Candidiasis is often observed in immunocompromised individuals such as HIV-infected patients.

Candida albicans is the infecting agent in 80 to 90 percent of patients.[6,7] *C albicans* is a diploid fungus that grows both as yeast and filmentous cells and a causal agent of opportunistc oral and genital infections in humans. Hospital-acquired infections by *C albicans* have become a cause of major health concern.

Recently, the frequency of non-albicans species (e.g., *Candida glabrata, , Candida krusei, Candida parapsilosis, and Candida tropicalis*) has increased, possibly secondary to greater use of over-the-counter antifungal products.[7] Non-*albicans* species of *Candida* account for a mere 5%–20% of the cases. Most of these infections with non-*albicans* species of *Candida* are due to *C glabrata* (5%–10% of cases) or *C tropicalis* (<5% of cases).[10,11] Non-albican Candida are commonly found in complicated cases of vaginal thrush such that first line treatment is ineffective. These cases are more likely in immunocompromised patients.[12] Candidiasis is often observed in immunocompromised individuals such as HIV-infected patients.

With the ever increasing population of immunocompromised individuals, trends have ***shown C glabrata*** to be a highly opportunistic pathogen of the urogenital tract, and of the bloodstream (*Candidemia*). It is especially prevalent in HIV positive people, and the elderly.[13]

C krusei is a rare but important cause of refractory vaginitis and is unique because of its intrinsic resistance to fluconazole.[14]

C parapsilosis is an infrequent isolate on vaginal cultures; its role as a vaginal pathogen remains unstudied. Although *C parapsilosis* is often a cause of vaginal symptoms, it seems to respond to a variety of antifungal agents and may even be a transient vaginal colonizer.[15]

In a majority of patients with candidal vulvovaginitis, drug therapy is convenient and effective. A small but significant group of patients remain symptomatic with recurrent, chronic candidiasis. A study of 805 patients revealed that the recurrence rate for ***C tropicalis*** was twice the rate for *C albicans*, and that despite continuous medical care and multiple therapies, the recurrent *C tropicalis* patients remained symptomatic with persistence of the organism. The difficulty encountered with eradication of *C tropicalis* may have been due to the lack of susceptibility of the cell membrane to the commonly used antifungal agents.[16]

Establishing Candida species as the cause of vaginitis can be difficult because as many as 50 percent of asymptomatic women have candidal organisms as part of their endogenous vaginal flora.[4]

TEST PRINCIPLE/ASSAY OVERVIEW

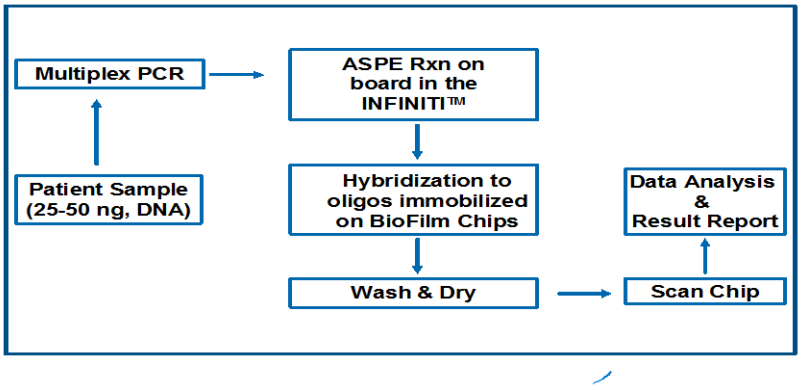
The INFINITI CV QUAD utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for multiplex detection of the presence of CV pathogens in deoxyribonucleic acid (DNA) obtained from cervical specimens.

The INFINITI CV QUAD is based on the following processes:

a) DNA extraction from endocervical and urine specimens
b) PCR amplification of purified DNA
c) Fluorescent label incorporation using analyte specific primer extension (ASPE)
d) Hybridization of the labeled ASPE primers to a microarray followed by washing.
e) Scanning of the microarray
f) Signal detection and analysis

Steps (c) through (f) are automated by the INFINITI Analyzer and INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI CV QUAD is an *in vitro* diagnostic device that consists of reagents and instrumentation which includes polymerase chain reaction (PCR) primers, microarrays, a thermal cycler, an imager, and software designed to detect the presence of the following pathogens: *Candida albicans* (CA), *Candida glabrata* (CG), *Candida krusei* (CK), *Candida parapsilosis* (CP), *and Candida tropicalis* (CT).

The INFINITI CV QUAD is comprised of the BioFilmChip® Microarray, the Intellipac® Reagent Module, and the PCR Amplification Mix. The instrumentation for the INFINITI CV QUAD is the AutoGenomics INFINITI Analyzer or the INFINITI PLUS Analyzer with the Qmatic™ Operating Software. The INFINITI Analyzer and the INFINITI PLUS Analyzer are CE marked.

The **BioFilmChip Microarray** consists of a polyester film coated with proprietary multi-layer components designed for DNA analysis. The layers have been designed to provide a versatile surface to enhance test performance. The INFINITI CV QUAD uses a microarray chip which has capture probes spotted on the surface of the film. Four (4) samples can be run on one microarray. Twelve (12) microarrays are housed in a magazine.

The **Intellipac Reagent Module** which acts as a communication link contains four reservoirs that house the test reagents and has an integrated memory chip. Reagent information such as lot number, expiration date, and volume usage are stored in the memory chip. The Intellipac Reagent Module communicates with the INFINITI Analyzer and provides the reagent information which appears on the assay report and printout. The Intellipac Reagent Management Module provides test reagent for 96 samples.

The **PCR Amplification Mix** consists of the reagents needed for the PCR amplification step of the assay. Each box of the PCR Amplification Mix provides 4 x 500µL vials of PCR Amplification

The **INFINITI Analyzer and INFINITI PLUS Analyzer** automate the INFINITI CV QUAD Assay and integrate all the discrete processes of sample (PCR reaction product) handling, reagent management, hybridization, detection, and results analysis. The assay is processed automatically and read by the built-in confocal microscope. Results are analyzed and presented as positive or negative for the presence of CV pathogens.

The Analyzers are provided with the Operator's Manuals. The Operator's Manual provides detailed description and operating principle of the Analyzers and instructions for use.

The INFINITI Analyzer and INFINITI PLUS Analyzer are CE marked

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- This test is to be used only with endocervical samples.
- To minimize the risk of cross contamination, sample preparation, PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as CLSI (Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline).
- All patient specimens are potentially hazardous and care should be taken when handling materials of human origin. No test method can offer complete assurance that HCV, HIV or other infectious agents are absent.
- Clean the lab and equipment with fresh 10% bleach or equivalent to prevent contamination.
  Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).
- Do not pool/mix reagents from different lots.
- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures

- Follow normal precautions for handling laboratory reagents. Do not mix reagents from different containers or from different lots.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves laboratory coats) and eye protection; do not eat, drink or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling
- Dispose of unused reagents, specimens and waste according to applicable country, federal, state and local regulations
- Safety data Sheets (SDS) are available upon request from AutoGenomics Customer Service

Sample Preparation
- Refer to the instructions provided with the sample processing kit.
- The PCR product cannot be stored prior to loading it onto the microarray. Use immediately**.**

INFINITI Analyzer and INFINITI PLUS Analyzer
- **Read the Operator's Manuals before operating the instruments.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered

STORAGE / STABILITY
BioFilmChip Microarray: 24 months Refrigerated (2 to 8°C)
Intellipac Reagent: 18 months Refrigerated (2 to 8°C)
Note: Remove the Intellipac from the Analyzer and store refrigerated as soon as possible. Do not use after Intellipac has been opened for four weeks.
Amplification Mix: 18 months Frozen (-30°C to -15°C)

Note: Specific product expiration date is printed on the product label

REAGENTS REQUIRED AND PROVIDED BY AUTOGENOMICS

ASSAY REAGENTS (SUFFICIENT FOR 192 TESTS)
- Catalog Number 04-1150-02 INFINITI CV QUAD Magazine – BioFilmChip® Microarray
  4 magazines per package; 48 tests per magazine
- Catalog Number 04-2150-02 INFINITI CV QUAD Intellipac® Reagent Management Module
  2 modules per package; 96 tests per module, 4 x 1.1 mL of DPE mix which contains:
  - dNTPs
  - Labeled -dCTP
  - Analyte Specific Primers
  - Extension reaction Buffer
- Catalog Number 04-3150-02 INFINITI CV QUAD Amp Mix: 4 x 500µL vials, 48 tests per vial, of PCR reaction master mix containing
  - dNTPs
  - Multiplex Primer Mix
  - $MgCl_2$
  - PCR Reaction Buffer

REAGENTS REQUIRED BUT NOT PROVIDED WITH THE ASSAY REAGENTS

- AutoGenomics Catalog Number 12-0470-02 INFINITI Sample processing Kit
- Internal Control 1 Catalog Number 12-0170-02
- AutoGenomics Catalog Number 12-0020-00 Wash Buffer
- AutoGenomics Catalog Number 12-0030-02 Hybridization Buffer.
- AutoGenomics Catalog Number 12-0280-02 CA DNA Template Control
- AutoGenomics Catalog Number 12-0290-02 CK DNA Template Control
- AutoGenomics Catalog Number 12-0300-02 CT DNA Template Control
- AutoGenomics Catalog Number 12-0310-02 CG DNA Template Control
- AutoGenomics Catalog Number 12-0320-02 CP DNA Template Control

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- Molecular Grade Water (DNAse and RNAse free)
- Platinum Taq DNA Polymerase (Invitrogen Catalog Number 10966-018)

EQUIPMENT

The following equipment is required but not provided with the assay reagents

- Pipettors
- Mini Centrifuge
- Pipette tips
- Microfuge tube Racks
- Thermocycler
- Vortex
- 0.2 ml thin wall tubes for PCR
- 1.5 ml microcentrifuge tubes
- 8-well Flat Strip Caps (Genesee Scientific, Catalog No. 22-623)
- AutoGenomics Product Number 11-0060-00: INFINITI Waste tray Stir Bars
- AutoGenomics Product Number 11-0020-00: INFINITI Waste Tray Liners
- AutoGenomics Product Number 11-0080-00: INFINITI Pipette Tips

- **FOR INFINITI Analyzer***:*
  - AutoGenomics Product Number 10-0010-99: INFINITI Analyzer
  - AutoGenomics Product Number 11-0030-00: 24-Well Plates with Lids
  - AutoGenomics Product Number 11-0050-00: INFINITI Temp Cycle Plate

- **FOR INFINITI PLUS Analyzer***:*
  - AutoGenomics Product Number 10-0020-99: INFINITI PLUS Analyzer
  - AutoGenomics Product Number 11-0100-00: 48-Well Plates and Product Number 11-0110-00: 48 Well Plate Lid

ASSAY PROCEDURE

Specimen Processing

It is recommended that the INFINITI Sample Processing Kit (Catalog Number 12-0470-00) be used for sample processing. Follow the instructions provided with the processing kit.

DNA Controls

It is required to run known positive controls and a negative control should also be included in each test run.

The following positive controls, available from AutoGenomics, are recommended for use with the INFINITI CV QUAD (refer to section on REAGENTS REQUIRED for Catalog Number)

(a) CA DNA Template Control
(b) CK DNA Template Control
(c) CT DNA Template Control
(d) CG DNA Template Control
(e) CP DNA Template Control

Note: Please use proper PCR technique to prevent contamination of reagents with positive controls. Sealing the 24WP containing sample DNA and "no template control" samples with caps **before** adding the positive controls is recommended to prevent cross contamination.

Amplification Reaction

Note:

- Keep Taq DNA polymerase on ice.
- Completely thaw reagents at room temperature then immediately place on ice.
- Vortex the amplification mix for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.
- To avoid potential contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach.
- Filter tips and gloves must be used when handling samples and controls.
- Make sure there is no abnormal evaporation of the PCR product. After PCR is complete, visually inspect for any volume change. All amplification reaction volumes should be about 15 µL. Otherwise, do not proceed with the assay.

Note:

- For the INFINTI Analyzer use the 24WP.
- For the INFINTI PLUS Analyzer use the 48WP.

1. Prepare the PCR master mix

Amplification mix	9.75 µL
Platinum Taq polymerase	0.25 µL
Internal Control 1	1.00 µL
Total volume of PCR master mix	11.0 µL

Note: Calculate the amount of each reagent needed based on the number of reactions.

2. Gently vortex the PCR master mix then dispense 11.0 µL of master mix into wells of the well plate.

3. Add 4.0 µL of sample or control DNA to each well

PCR master mix	11.0 µL
Sample	4.0 µL
Total volume of amplification reaction	15.0 µL

Note: **This is a QUAD assay**. When loading samples, always load the samples in multiples of four and in consecutive order. For example, if loading 8 samples, load wells A1 to A8. **Do not** load the B wells**.**

4. Place the well plate, sealed with 8-well flat strip caps, in a thermocycler and immediately commence the amplification reaction using the following program.

Step No.	Temperature °C	Time (sec)	No. of Cycles
1	94	120	1
2	94 63-55 (-0.8/cycle) 72	10 60 40	10x
3	94 55 72	10 60 40	30x
4	4	hold	1

Note: After each cycle in step 2, the temperature is decreased by 0.8°C. When an Eppendorf Mastercycler EP was used with the ramp rate set at 75%, the total cycling time was one (1) hours and 44 minutes (± 5 min). If using other thermocycler models, we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time.

Sample Loading

1) Carefully remove the 8-well flat strip caps to avoid splashing.
2) Load the well plate in the appropriate orientation (with well A1 in the back left corner) into the Analyzer
   - **INFINITI Analyzer:** Load the assembled 24WP with the associated lid (Catalog # 11-0030-00).
   - **INFINITI PLUS Analyzer:** Load the assembled 48WP with a clean 48WP lid (see instructions in the INFINITI PLUS Analyzer Operator's Manual) (Catalog # 11-0110-00, reusable).
3) Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffer.
   - **FOR INFINITI Analyzer***:*
     Wash buffer and hybridization buffer be placed in the INFINITI bottle holders. Wash Buffer goes in the left holder (near the magazine) and Hybridization buffer 2 in the right holder (near the Intellipac).
   - **FOR INFINITI PLUS Analyzer***:*
     Hybridization Buffer 2 should be placed in the INFINITI bottle holders. Hybridization Buffer 2 goes in the right holder (near the Intellipac).

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI Analyzer Operator's Manual (Part Number EM-34000)
INFINITI PLUS Analyzer Operator's Manual (Part Number EM-34041)

QUALITY CONTROL

- Maintain calibration of thermocycler according to manufacturer's specifications.
- Maintain calibration of INFINITI or INFINITI PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

LIMITATIONS

The results obtained from the INFINITI CV QUAD should be used and interpreted only in the context of the overall clinical diagnosis. AutoGenomics is not responsible for any clinical decisions that are made.

INTERPRETATION OF RESULTS

Results from the INFINITI CV QUAD are reported to the user as "Positive" or "Negative" for the presence of pathogens detected.

The Internal Control is intended to identify specimens that contain polymerase inhibitors. INFINITI CV QUAD results are interpreted as follows:

Analyte Result	Internal Control (IC) Result	INTERPRETATION
All Analytes Negative	Positive	Negative
All Analytes Negative	Negative	Invalid Test Result
Any Analyte Positive	Positive or Negative	Valid Test Result

If the analyzer detects a problem or an error (e.g., assay parameters not met), no results will be reported. Instead, a description of the problem or error code will be displayed. The Trouble Shooting section of the INFINITI Analyzer Operator's Manual provides an explanation of the errors. The assay needs to be repeated.

DISPOSAL

Waste materials for the INFINITI CV QUAD are common waste materials generated in clinical laboratories, and should be handled/ disposed of in accordance with the policies/procedures in place in the clinical laboratory.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Studies related to specificity were conducted during assay development. PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. ASP primer specificity was determined by the correct calls made by the assay using known genomic samples. Capture probe specificity was determined by hybridizing different oligos and demonstrating that correct oligo hybridizes to the known spot.

Analytical Specificity/Cross-Reactivity study was performed to evaluate the effect of the presence of known microbial DNA on the INFINITI CV QUAD Assay. The study demonstrated no cross-reactivity of the INFINITI CV QUAD Assay with microbial DNA listed in Table 1.

Table 1

Escherichia coli	*Corynebacterium striatum*
Klebsiella pneumoniae	*Peptostreptococcus anaerobis*
Pseudomonas aeruginosa	*Clostridium sordelli*
Haemophilus influenzae	*Clostridium difficile*
Proteus vulgaris	*Bacteriodes fragilis*
Enterobacter cloacae	*Bacteriodes uniformis*
Acinetobacter baumannii	*Fusobacterium nucleatum*
Moraxella (Branhamella) catarrhalis	*Mobiluncus mulieris*
Neisseria meningitidis	*Mobiluncus curtisii*
Streptococcus agalactiae	*Gardenella vaginalis*
Enterococcus (Streptococcus) faecalis	*Prevotella bivia*
Streptococcus pyogenes	*Atopobium vaginae*
Staphylococcus aureus	*Lactobacillus jensenii*
Staphylococcus epidermidis	*Neisseria gonorrhoeae*

Analytical Sensitivity (Level of Detection)

A study was performed to establish the working range for the INFINITI CV QUAD Assay. The working range is the upper and lower DNA input per test where the INFINITI CV QUAD Assay gives at least 90% correct calls. The following table provides the working range for each analyst in the INFINITI CV QUAD Assay.

Analyte	Working Range (copies/test)
Candida albicans (CA)	500 – 2,000
Candida glabrata (CG)	500 – 2,000
Candida krusei (CK)	500 – 2,000
Candida parapsilosis (CP)	4,000 – 16,000
Candida tropicalis (CT)	2,000 – 8,000

.

Sample Carry-over

Sample carryover studies using plasmid samples demonstrated that there is no sample carry-over with the INFINITI CV QUAD Assay. *Candida albicans (CA*) and *Candida krusei (CK)* Plasmid DNA samples and water were run alternatively to determine if one sample will carry over to next. No carry-over contamination was observed.

Potential Interference from Blood and other Substances

Substance interference studies indicated that the INFINITI CV QUAD Assay will not be affected by the presence of products commonly used in the vaginal tract: vaginal cream, vaginal ointment, douche, and contraceptive gel at concentrations typically found in the vaginal tract.

Results of the interference studies using whole blood at a concentration of 5% indicated that bloody specimens may interfere with assay performance.

Precision and Reproducibility

A reproducibility study was performed to demonstrate the reproducibility of the INFINITI CV QUAD Assay. Five controls representing the target pathogens were used as samples for the test. The study involved six lots and three instruments; 4 replicates per sample. A total of 120 tests were performed with 112 correct calls (93.3%). Investigation on the failure indicated that the failure was not related to the assay performance.

The reproducibility study demonstrated lot-to-lot and instrument-to-instrument reproducibility of the INFINITI CV QUAD Assay.

Clinical Validation Studies

A study was performed demonstrate that the INFINITI CV QUAD Assay works on clinical samples. Clinical samples used in this study were pre-characterized (tested) by an independent laboratory (3rd party) using the standard laboratory method (Comparator Method).

The pre-characterized samples were tested by two sites using the INFINITI CV QUAD Assay. The clinical validation study demonstrated a 96% agreement with the Comparator Method. There were two (2) false negative results: one was for the CA sample, the other was for a CK sample.

Sample Type(a)	# Samples	INFINITI CV QUAD Results						
		Negative	CA	CG	CK	CP	CT	total
Negative	42	44						44
CA	4		3					3
CG	2			2				2
CK	4				3			3
CP	2					2		2
CY	2						2	2
total	56							56

(a) Based on 3rd party standard laboratory method

REFERENCES

1. Kent HL. *Epidemiology of vaginitis*. Am J Obstet Gynecol. 1991;165:1168–76.
2. Monif GR. *Classification and pathogenesis of vulvovaginal candidiasis*. Am J Obstet Gynecol. 1985;152:935–9.
3. Foxman B. *The epidemiology of vulvovaginal candidiasis: risk factors*. Am J Public Health. 1990;80:329–31.
4. Sobel JD. *Candidal vulvovaginitis.* Clin Obstet Gynecol. 1993;36:153–65.
5. Geiger AM, Foxman B. *Risk factors for vulvovaginal candidiasis: a case-control study among university students.* Epidemiology. 1996;7:182–7.
6. Sobel JD. *Vaginitis.* N Engl J Med. 1997;337:1896–903.
7. Horowitz BJ, Giaquinta D, Ito S. *Evolving pathogens in vulvovaginal candidiasis: implications for patient care.* J Clin Pharmacol. 1992;32:248–55.
8. Ryan KJ, Ray CG (editors) (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill. ISBN 0-8385-8529-9.
9. Enfert C; Hube B (editors) (2007). *Candida: Comparative and Functional Genomics*. Caister Academic Press. ISBN 978-1-904455-13-4.
10. Sobel JD . *Pathogenesis and epidemiology of vulvovaginal candidiasis*. *Ann N Y Acad Sci* 1988;544:547-57.
11. Grillot R . *Les mycoses humaines: demarche diagnostique*. Paris*:* Elsevier*;* 1996. Mycosis genitals*; p.* 116-24.
12. Sobel, Jack. *Vulvovaginal Candidiasis*. UpToDate. Retrieved 26 February 2012.
13. Fidel F, Vazquez J, Sobel J. (1999). *Candida glabrata*: Review of Epidemiology, Pathogenesis, and Clinical Disease with Comparison to *C. albicans*. Clinical Microbiology Reviews **12** (1): 80–96. PMC 88907. PMID 9880475
14. Singh S, Sobel JD, Bhargava P, Boikov D, Vasques JA. *Vaginits Due to Candida krusei: Epidemiology, Clinical Aspects, and Therapy* http://cid.oxfordjournals.org/content/35/9/1066.
15. Nyirjesy P, Alexander AB, Weitz MV. *Vaginal Candida parapsilosis: pathogen or bystander?* Onfect Dis Obstet Gynecol. 2005 march; 13(1): 37-41
16. Harowitz BJ, Edelstein S, Lippman L. *Candidatropicalis Vulvovaginitis.* Obstet Gynecol. 1985 august