PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

# Yeast Identification Procedure

**Department of Microbiology**

**Effective date**: 05/19/2000

**Last reviewed**: 1/3/2013

## Table of Contents

# 1.0 Purpose

Yeasts are a heterogenous group of fungi. Identification of clinical isolates is commonly based on phenotypic characteristics. Morphology is used primarily to establish the genus. Colony morphology can be assessed on routine culture media such as TSA with 5% Sheep Blood Agar, Chocolate Agar, or Sabouraud Dextrose Agar. The use of a CHROMagar Candida permits the differentiation of certain species of *Candida*. After 48 h incubation, *C. albicans*, *C. krusei*, and *C. tropicalis* produce unique colonies that permit identification without further testing. Other *Candida* species require further testing for identification. Due to the differences in morphology and colors of yeast colonies, this medium also facilitates the detection of mixed yeast cultures. Microscopic morphology may be assessed by a simple wet prep exam. *Candida* species typically produce oval or elongated blastoconidia with or without pseudohyphae. *Cryptococcus neoformans* and *Cryptococcus gattii* appear as round, dark-walled cells of various sizes with no pseudohyphae. Other yeast may produce unique microscopic structures which can be observed by subculture to a morphology medium such as Corn Meal with Tween 80.

Morphologic observations should be combined with biochemical testing to provide genus confirmation and to differentiate various species when necessary. Biochemical testing may be comprised of traditional manual tests or a panel of tests which are interpreted by an automated system.

# 2.0 Clinical Significance

The extent to which yeast isolates need to be identified depends primarily on the body site from which they are isolated and the clinical relevancy of full identification. The most common yeasts associated with infection are *Candida* species. *Candida albicans* can be rapidly differentiated from other species of *Candida* by performing a germ tube test. For many specimen types, it is sufficient to report *Candida albicans* or *Candida* not *albicans* based on the microscopic morphology and the results of the germ tube test. Even that level of identification is not usually necessary for isolates from the lower respiratory tract. *Candida* species are seldom of clinical significance in the lower respiratory tract, and diagnosis of infection is based on histologic examination of lung biopsy material, not culture. *Cryptococcus neoformans* and *Cryptococcus gattii* are typically the only yeast of concern in lower respiratory specimens (aside from the yeast forms of thermally dimorphic fungi). *C. neoformans* and *C. gattii* can usually be rapidly ruled out by colony and microscopic morphology. For isolates from sterile body fluids and tissues, a full identification should be performed as therapeutic regimens may be impacted by the identification. Work-up of these isolates should begin with observing colony and microscopic morphology, followed by appropriate confirmatory biochemical testing described below.

# 3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained on all identification tests. In some cases, yeast isolates may be identified on the routine Bacteriology bench. However, uncommon isolates should be evaluated with Rounds consultation and referred to the Mycology lab for additional testing if necessary. Testing includes but is not limited to: manual test performance and result interpretation, automated instrument operation, QC checks, administrative tasks and record keeping of information vital to verification of isolate identification. Technical proficiency is assessed with biannual CAP surveys. Records of proficiency test results are to be kept within the employee's competency record in the department. Performance reviews of technical personnel are to be carried out annually.

# 4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All isolates must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

**This procedure may expose you to:**

- Bloodborne pathogens (Germ Tube Plasma – human plasma)
- Airborne pathogens

**To perform this procedure, you must use:**

- Gloves (when handling specimens or Germ Tube Plasma)
- Laboratory Coat
- Biological safety cabinet (for mucoid colonies or colonies with aerial hyphae)

**Disinfectant following procedure:**

- Diluted bleach (10% solution made fresh daily)

# 5.0 Specimen Requirements

Yeast colonies should be well isolated when performing screening and confirmatory testing. Yeast may be differentiated by colony or microscopic morphology. Mixed yeast cultures may be more readily recognized when grown on CHROMagar Candida.

# 6.0 Materials Required

## 6.1 Equipment and/or Testing System

- Aerobic incubator at 42 ± 2˚C
- Aerobic incubator at 25 to 30˚C
- BD Phoenix™ Automated Microbiology System
- BD Epicenter™ software
- BD PhoenixSpec™ Nephelometer
- BD Phoenix™ Inoculation Station

## 6.2 Consumables

- Wooden applicators
- Sterile inoculation loops
- Sterile cotton swabs
- Glass slides
- Coverslips

## 6.3 Media & Reagents

Media and reagents should be stored at 2 - 8˚C unless otherwise indicated.

- Sterile saline. Do not use if there appears to be evidence of contamination (i.e., haziness, turbidity).
- Germ Tube Plasma (human plasma obtained from blood bank). Store aliquots at -20 ± 2˚C until needed. Thaw at room temperature.
- Sabouraud Dextrose Agar (BD Catalog Number 221180)
- CHROMagar Candida Agar (BD Catalog Number 254093)
- Corn Meal with Tween 80 Agar (Hardy Diagnostics Catalog Number W10)
- Urea Agar slants (BD Catalog Number 221097)
- Rapid Trehalose Assimilation Broth (Remel Catalog Number R064856)
- Bird Seed Agar (BD Catalog Number 297875)
- CGB Agar (Hardy Diagnostics Catalog Number G113)
- Caffeic Acid Disks (Hardy Diagnostics Catalog Number Z118)
- Phoenix™ Yeast ID Panel (BD Catalog Number 448316), 25 panels. Store at 15 - 25˚C. Do not use panel if desiccant is missing or if the desiccant pouch is torn. Panels must be used within 2 h of being removed from the pouch.
- Phoenix™ ID Broth (BD Catalog Number 246001), 100, 4.5 mL tubes. Store at 2-25˚C. Visually inspect the tubes for cracks, leaks, etc. Do not use if there appears to be a leak, tube or cap damage or evidence of contamination (i.e., haziness, turbidity).

## 6.4 Control and Calibration Materials

- Phoenix™ Calibrator Tube Set. Store at 15 - 30˚C until expiration date.
- Refer to individual test procedures and/or the QC Reference Guide for specific control strains and instructions for reagent and media QC.

# 7.0 Identification Procedures

The identification procedures below focus on the most frequently encountered and clinically relevant yeasts, including *Candida* and *Cryptococcus.* Other genera of yeast may be recovered from clinical specimens and may represent opportunistic infection. These isolates should be evaluated with Rounds consultation. Other genera of yeast may present with distinguishing features that help separate them. For example, salmon- or red-pigmented colonies are characteristic of *Rhodotorula* and *Sporobolomyces* spp. Microscopic morphology may also distinguish other genera. Observing arthroconidia suggests *Trichosporon* spp., *Blastoschizomyces capitus,* or *Geotrichum candidum.*

## 7.1 Routine Bacterial Cultures

### 7.1.1 Lower Respiratory Cultures

Yeast isolates from sputum and bronchial washings should not be worked up unless the colonies appear mucoid. Yeast from BAL, bronchial brush, lung, or transtracheal aspirate specimens should be examined microscopically. *Candida* species are most commonly encountered and produce oval or elongated cells, with or without pseudohyphae. A Germ Tube Test should be performed on these isolates. Only *C. albicans* (and *C. dubliniensis*) will produce germ tubes in plasma within 3 h. The isolate can be identified as *C. albicans* or *Candida* not *albicans.*

**Figure 1**
**Positive Germ tube**

**Figure 2**
**Negative Germ Tube**

**The germ tube is the initial growth of true hyphae. It grows out of the cell and the base is not constricted (Figure 1), unlike pseudohyphae which are elongated buds (Figure 2).**

*Cryptococcus neoformans* and *Cryptococcus gattii* appear as round, dark-walled cells of various sizes when examined in a wet prep (Figure 3). *Cryptococcus* may produce mucoid colonies (Figure 4), but this is not always apparent until after several days of incubation and may vary on different media. Yeast isolates that are suggestive of *Cryptococcus* should be brought up on Rounds and referred to the Mycology lab for further testing.

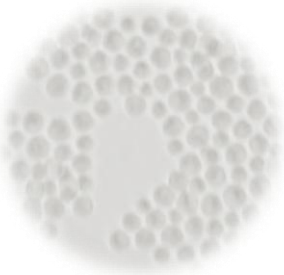


**Figure 3**
**Microscopic morphology of *C. neoformans***

**Figure 4**
**Colony morphology of *C. neoformans***

### 7.1.2 Urine Cultures

Yeast isolates from urine should be worked up in accordance with the Urine Culture Procedure based on colony count. Urine isolates may be identified as *C. albicans* or *Candida* not *albicans* based on the germ tube test result. Yeast isolates that do not resemble *Candida* species microscopically should be brought up on Rounds. While *Candida* species comprise the vast majority of yeast associated with UTI, other yeast may be implicated. There are rare case reports of *Cryptococcus neoformans* isolated from urine of immunocompromised patients.

### 7.1.3 Wound Cultures

Yeast are considered potential pathogens when recovered from wound cultures. However, they may also represent colonization and work-up should be driven by the Q-score as with other potential pathogens. When identification is warranted based on the Q-score, yeast isolates may be identified as *C. albicans* or *Candida* not *albicans* based on the germ tube test result. Yeast isolates that do not resemble *Candida* species microscopically should be brought up on Rounds. If the potential pathogens exceed the Q-score, and correlation does not dictate work-up, isolates should be reported generically as "Yeast" without additional testing.

### 7.1.4 Sterile Body Fluid and Tissue Cultures

Yeast from sterile body sites such as body fluids and tissue specimens should be identified to the genus and species level. Identification should begin with a wet prep to observe microscopic morphology. If round cells are observed, the isolate should be reviewed on Rounds and referred to the Mycology lab for further testing. If oval or elongated cells are observed, the germ tube test should be performed to rule out *C. albicans.* If the germ tube test is negative, and the colony is small and relatively slow growing, and the wet prep shows small, oval cells, perform the Rapid Trehalose Assimilation test for identification of *C. glabrata* (Figures 5 & 6).

**Figure 5**
**Microscopic morphology of *C. glabrata***

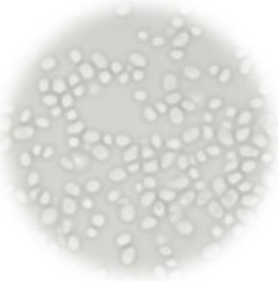


**Figure 6**
**Positive Rapid Trehalose**

If the isolate is not *C. albicans* or *C. glabrata*, identification should be performed using the Phoenix™ Yeast ID panel on the Phoenix™ Automated Microbiology System. Corn Meal Tween 80 agar should also be inoculated to provide an accurate picture of the microscopic morphology.

### 7.1.5 Blood Cultures

Yeast isolates from positive blood cultures should also be identified to the genus and species level. However, work-up is aided by subculturing the positive bottle to CHROMagar Candida after yeast are first observed in the Gram stain. It is not uncommon for blood cultures to grow more than one type of yeast. The CHROMagar medium helps to differentiate mixed cultures. After 48 h incubation, *C. albicans*, *C. krusei*, and *C. tropicalis* produce unique colonies that permit identification without further testing. Other yeast may produce white, pale pink, or dark pink colonies and further testing is required. A wet prep should be performed to observe microscopic morphology. If round cells are observed, the isolate should be reviewed on Rounds and referred to the Mycology lab for further testing. If the colonies are small and relatively slow growing, and the wet prep shows small, oval cells, perform the Rapid Trehalose Assimilation test for identification of *C. glabrata* (Figures 5 & 6). If the isolate is not *C. glabrata*, identification should be performed using the Phoenix™ Yeast ID panel on the Phoenix™ Automated Microbiology System. Corn Meal Tween 80 agar should also be inoculated to provide an accurate picture of the microscopic morphology.

# Routine Bacterial Culture Yeast Identification

**Routine Culture**
Sterile Body Fluid, Tissue
Wound/Abscess, BAL, Bronch Brush, Lung Aspirate, Urine

**Culture, Genital**

**Culture, Blood**

Perform Wet Prep

Report **Yeast**

CHROM

Non-ID

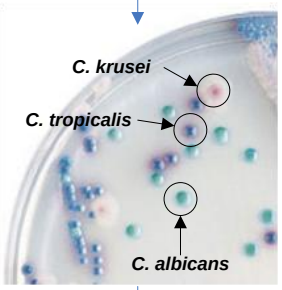
Perform Wet Prep

Oval or elongated cells

Round yeast

Other

Small, oval yeast




Report ID

Germ Tube

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BAL, Bronch Brush, Urine, Wound

Report ***Candida* not *albicans***

Sterile Body Fluid, Tissue

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Report ***C. albicans***

Cell morphology

Small, oval yeast

Other

Additional Testing
Phoenix YEAST ID
Caffeic Acid Disk
Urea Slant
Cornmeal Tween 80
Birdseed Agar
CGB Agar

**Refer testing to Mycology**

Rapid Trehalose

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Report ***C. glabrata***

Report ***C. glabrata***

\+

Rapid Trehalose

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Additional Testing
Phoenix YEAST ID
Cornmeal Tween 80

## 7.2 Fungus Cultures & Yeast Screens

### 7.2.1 Lower Respiratory Fungus Cultures

Work-up of yeast isolates from lower respiratory specimens is aided by the addition of CHROMagar Candida in the primary set-up. After 48 h incubation, identification of *C. albicans, C. krusei,* or *C. tropicalis* can be made based on their unique colony morphology. However, if there are less than 5 colonies present in sputum or bronchial washing cultures, a generic report of "Mixed Flora" should be issued. This does not apply to BAL, bronchial brush, transtracheal aspirate, or lung specimens. Identification of isolates from these specimens should be reported regardless of the number of colonies present.

Yeast that cannot be identified on CHROMagar should be evaluated by performing a wet prep regardless of the number of colonies present. If round yeast cells are observed, additional testing should be performed to rule out *Cryptococcus*. The isolate should be tested using a Phoenix™ Yeast ID panel and subcultured to a Urea Agar slant, Bird Seed Agar plate, CGB Agar plate, and Cornmeal with Tween 80 Agar plate. A Caffeic Acid Disk test can be performed from the growth on Cornmeal Tween 80 plate to provide more rapid results than the Bird Seed Agar. If the disk test is negative, the final result should be interpreted from the Bird Seed Agar plate. Identification of *Cryptococcus* should include results from the Phoenix™ instrument as well as the conventional testing. Consult Rounds for all suspected *Cryptococcus* isolates.

### 7.2.2 Wound Fungus Cultures

Yeast isolates from "wound" type specimens should be evaluated first by performing a wet prep to examine colony morphology. Isolates that produce elongated or oval cells consistent with *Candida* should be characterized as *C. albicans* or *Candida* not *albicans* based on the results of the Germ Tube test. Isolates that produce round cells should be investigated to rule out *Cryptococcus* as described above for isolates from lower respiratory tract specimens.

### 7.2.3 Sterile Body Fluid and Tissue Fungus Cultures

Yeast from sterile body sites such as body fluids and tissue specimens should be identified to the genus and species level. Identification should begin with a wet prep to observe microscopic morphology. If round cells are observed, the isolate should be reviewed on Rounds and investigated to rule out *Cryptococcus,* as above for isolates from lower respiratory specimens. If oval or elongated cells are observed, the Germ Tube test should be performed to rule out *C. albicans.* If the Germ Tube test is negative, and the colony is small and relatively slow growing, and the wet prep shows small, oval cells, perform the Rapid Trehalose Assimilation test for identification of *C. glabrata* (Figures 5 & 6). If the isolate is not *C. albicans* or *C. glabrata*, identification should be performed using the Phoenix™ Yeast ID panel on the Phoenix™ Automated Microbiology System. Corn Meal Tween 80 agar should also be inoculated to provide an accurate picture of the microscopic morphology.

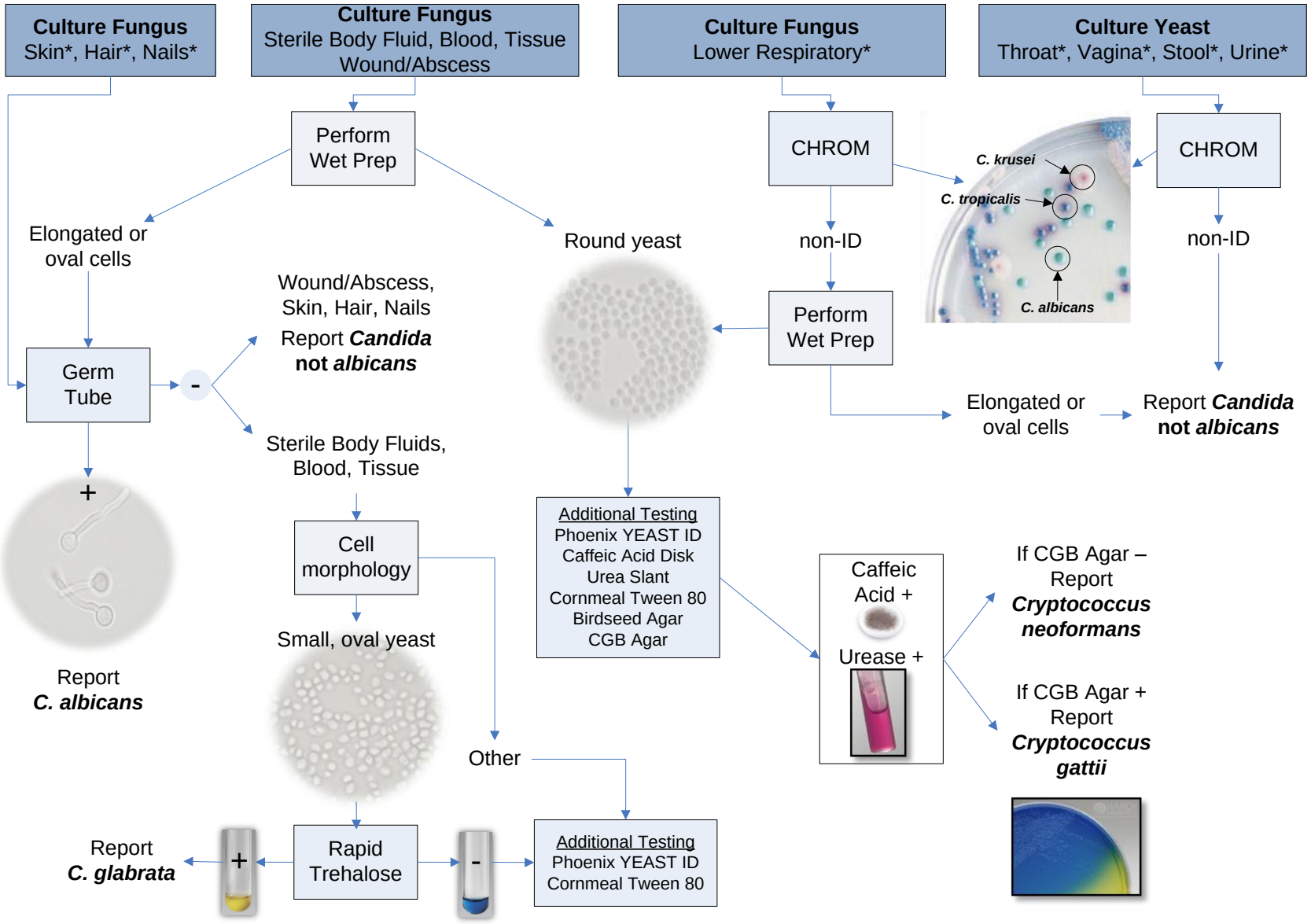
### 7.2.4 Skin, Hair, and Nail Fungus Cultures

If 5 or more colonies are present, perform a wet prep. If elongated or oval cells are seen, perform a Germ Tube test. Yeast may be identified and reported as *C. albicans* or *Candida* not *albicans* based on the germ tube test result. If the microscopic morphology is not consistent with *Candida*, or if there are less than 5 colonies growing, consult Rounds.

### 7.2.5 Genital, Stool, Throat, and Urine Cultures for Yeast

Yeast cultures ordered on these sources should be inoculated on CHROMagar Candida. After 48 h incubation, identification of *C. albicans, C. krusei,* or *C. tropicalis* can be made based on their unique colony morphologies. Yeast that cannot be identified on CHROMagar may be reported as *Candida* not *albicans.* However, a wet prep should be performed to ensure that the cells are consistent with *Candida*. Questionable isolates should be reviewed during Rounds. If round yeast cells are observed from urine isolates, additional testing should be performed to rule out *Cryptococcus*. If less than 5 colonies of yeast are growing, or < 10,000 CFU/mL from urine, consult Rounds.

# Fungus Culture Yeast Identification




*If < 5 colonies present for sources indicated, refer to procedure.

## 8.0 Interpretation of Results

Refer to individual test procedures for specific information.

## 9.0 Quality Control & Quality Assurance

Refer to individual test procedures for specific information. Unusual or infrequently encountered identifications and all *Cryptococcus* isolates should be reviewed on Rounds.

## 10.0 Limitations

1. Refer to individual test procedures for specific information.

## 11.0 References


1. Larone, D.H. 2011. Medically Important Fungi: A Guide to Identification, 5th ed., ASM Press, Washington, D.C.
2. Versalovic, J, K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry, D. W. Warnock. 2011. Manual of Clinical Microbiology, 10th ed., Vol. 1, ASM Press, Washington, D.C.
3. http://www.medtraining.org/ Mycology Training module.
4. http://hardydiagnostics.com/ online technical information.
5. http://www.bd.com/ds/productCenter/ online technical information.
6. http://www.remel.com/Clinical/Microbiology.aspx online technical information.


## 12.0 Document Control

Adopted/Reviewed by director (AR) and supervisor (JC) 5/19/2000.

Reviewed by JC: 6/14/01, 4/1/02, 3/2003, 4/2004, 6/2005, 6/2006, 6/2007, 5/2008, 7/2009, 4/1/2011

Reviewed by J. Schappert 7/10/10

Revised: 5/15/12 Updated to eliminate Remel UniYeast Tek and include Phoenix Yeast ID. 12/28/12 Updated procedure with flowcharts and detailed identification protocols based on isolate source.