**PROCEDURE: PNA FISH**

1. **PRINCIPLE**

PNA FISH test uses fluorescent labeled PNA (peptide nucleic acid) probes in a highly sensitive and specific fluorescence *in situ* hybridization (FISH) assay targeting species-specific rRNA in yeast.

1. **AVAILABILITY**

Test will be performed Sunday through Saturday 7:30 AM – 2:30 PM.

Monday – Friday test will be done in Mycology. On weekends a person from the Mycology team will perform the test.

1. **TEST CODE**

See Appendix for Soft codes

1. **SPECIMEN COLLECTION AND PROCESSING**

Blood cultures positive for yeast by gram stain will be eligible for further staining with PNA FISH. Once a patient is positive additional positives will be sent to Mycology to determine if the organism is morphologically the same without the use of additional probes.

1. **EQUIPMENT AND MATERIALS**
	1. PNA FISH slides and coverslips
	2. Glass dishes and slides holders
	3. Heating block and water bath
	4. Fixation solution
	5. PNA probe
	6. Wash solution
	7. Deionized water
	8. Mounting medium
	9. Fluorescent microscope with Texas Red filter
2. **PROCEDURE**
	1. For blood cultures positive for yeast by gram stain, prepare a slide for PNA FISH by putting one drop of fixation solution and one drop of blood on a PNA slide. Make two slides in the same manner.
	2. Allow smears to dry and methanol fix them by overlaying the slide with methanol and allowing it to evaporate.
	3. Prepare fresh wash solution by putting 4 ml of 60x wash solution into staining dish followed by 240 ml deionized or distilled water. Cover dish and place in water bath with cover. It takes approximately 30 minutes for the bath to reach 55°C.
	4. Add one drop of PNA dual probe reagent (*C. albicans/glabrata*) to slide, coverslip and push down to avoid air bubbles. Using a dual probe control slide, add one drop each of PNA reagent to positive and negative wells and coverslip. Incubate slides for 30 + 5 minutes at 55 +1°C on heat block.
	5. Immerse slides in preheated wash solution at 55+1°C. Coverslips should fall off on their own but if they do not gently push off with forceps. Excess agitation may result in cross contamination by yeast on the control slide or by yeast from positive slides if more than one patient is stained at the same time.
	6. Incubate slides in bath for 30 + 5 minutes.
	7. Allow slides to air dry.
	8. Add 1 drop Mounting Medium to slide; coverslip and gently push down to avoid bubbles.
	9. Examine within 2 hours using the 40X objective of a fluorescent microscope equipped with a Texas Red filter.
	10. If the dual probe slide is negative repeat the procedure using the traffic light probe.
3. **QUALITY CONTROL**
	1. Positive and negative control slides are run with each test performed.
4. **TEST INTERPRETATION**
	1. For dual probe C. albicans stains green and C. glabrata stains red.
	2. If yeast does not fluoresce by dual probe perform traffic light probe using the same methodology. C. parapsilosis stains green, C. tropicalis stains yellow and C. krusei stains red. Traffic light controls are also available for use.

Dual probe positive

Candida albicans = green

Candida glabrata = red

Traffic light probe positive

Candida albicans/parapsilosis = green

Candida glabrata/krusei = red

Candida tropicalis= yellow

1. **REPORTING RESULTS**
	1. Dual probe positive:
		1. Green = Candida albicans
		2. Red = Candida glabrata
	2. Dual probe negative, Traffic light probe positive:
		1. Green = Candida parapsilosis
		2. Red = Candida krusei
		3. Yellow = Candida tropicalis
	3. Call results to physician.
2. **LIMITATIONS**
	1. All yeast negative by probe must be identified using Vitek MS (MALDI) or Vitek 2 yeast cards.
	2. Inconclusive results should be confirmed with Vitek MS (MALDI) or Vitek 2 yeast card.
	3. Presence of more than one yeast by color may mean cross contamination from positive control and should not be reported.
	4. *C. africana* (novel *Candida* strain) has not been evaluated by the company and therefore the performance data has not been established.
3. **REFERENCES**
	1. Merz, W.G. and M. Gherna. 2009. A rapid PNA FISH for the direct species-specific identification of *Candida* species from positive blood bottles in less than 1.5 hours. ASM General Meeting. F-055.
	2. Reller, M.E., A.B. Mallonee, N.P. Kwatkowski and W.G. Merz. 2007. Use of PNA FISH for definitive, rapid identification of five common species of *Candida*. J. Clin. Microbiol. 45:3802-3803.
	3. Rigby, S., G.W. Procop, G. Hasse, D. Wilson, G. Hall, C. Kurtzman,
	4. K. Oliveira, S. Von Oy, J.J. Hyldig-Nielsen, J. Couli, and H. Stender. 2002. Fluorescence in situ hybridization with peptide nucleic acid probes for rapid identification of *Candida albicans* directly from blood culture bottles. J. Clin. Microbiol. 40:2182-2186.
	5. Shepard, J.R.,R.M. Addison, B.D. Alexander, P. Della-Latta,
	6. M. Gherna, G. Haase, G. Hall, J.K. Johnson, W.G. Merz, H. Peltroche-Llacsahuanga, H. Stender, R. Venezia, D. Wilson, G.W. Procop, F. Wu and M.J. Fiandaca. 2008. Multicenter evaluation of the *Candida albicans/Candida glabrata* PNA FISH method for simultaneous dual color identification of *Candida albicans* and *Candida glabrata* directly from blood culture bottles. J. Clin. Microbiol. 46:50-55
4. **REVISIONS**
	1. December 10, 2019 – Changed method of fixing slides from heat fixation to methanol fixation.