JOURNAL OF CLINICAL MICROBIOLOGY, June 1996, p. 1583–1585 Vol. 34, No. 6
0095-1137/96/$04.00+0

Duration of Incubation of Fungal Cultures

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Received 8 December 1995/Returned for modification 6 January 1996/Accepted 13 March 1996

To determine the optimum duration of incubation for recovery of fungi, the results of 2,173 consecutive clinical cultures were reviewed. Overall, 94% of fungal isolates were detected by day 7 and 98% were detected by day 14. Yeasts were usually (98%) detected within the first week of incubation. Recovery of molds required more time, but 81% were detected by day 7 and more than 96% were detected by day 14.

Clinical mycology laboratories have traditionally held routine cultures for 4 weeks or more in order to maximize recovery of slowly growing fungi (1–4). This practice is based on experience with a mixture of specimen types different from that encountered in many contemporary hospital laboratories and on the use of older medium formulations. In our hospital many fungal culture specimens derive from inpatients with suspected nosocomial infections, and our experience suggested that for most specimen types, prolonged incubation did little to increase recovery of clinically relevant organisms but greatly increased the laboratory work load. In order to determine the optimum incubation period for routine fungal cultures, we recorded the time to initial detection of growth for all fungal isolates during a 6-month period and analyzed the results according to specimen and organism type.

Specimen characteristics. All fungal specimens submitted from January to June 1992 to the clinical mycology laboratory at Duke University Medical Center, a 1,125-bed teaching and referral hospital in central North Carolina, were included in the analysis. A total of 2,173 specimens were submitted from 1,207 patients (average, 1.8 specimens per patient). Physicians had explicitly requested fungal culture for the majority of specimens (94%); for the remainder, fungal growth was observed in bacterial culture and referred to the mycology laboratory for workup. Most specimens (1,738 of 2,173; 80%) derived from inpatients; of these, over half came from patients hospitalized for more than 1 week. The most frequent specimen types and numbers were as follows: respiratory tract samples (including sputum, bronchoalveolar lavage, bronchial brushing, and endotracheal aspirate specimens), 547 (25%); cerebrospinal fluids, 343 (16%); tissue specimens and sterile aspirates, 302 (14%); body fluids (including pleural, peritoneal, and synovial fluids), 285 (13%); urine specimens, 199 (9%); stool specimens, 178 (8%); and miscellaneous specimen types (including superficial swabs and sinonasal and oropharyngeal specimens), 319 (15%). Routine broth blood cultures for fungus were not included in this analysis since they were performed on automated instruments in a different section of our laboratory; a small number of blood samples (34) received in Isolator (Wampole Laboratories, Cranbury, N.J.) tubes were included in the miscellaneous specimen group.

Culture methods. Tissues were sterilely minced. Body fluid specimens over 2 ml in volume were concentrated by centrifugation. For the majority of specimens, three plates were inoculated: inhibitory mold agar, brain heart infusion agar (BHIA), and BHIA supplemented with 10% sheep blood, gentamicin, and chloramphenicol (BHIBA) (Becton Dickinson Microbiology Systems, Cockeysville, Md.). For stool and urine specimens the BHIA plate was omitted. For cerebrospinal fluid specimens the BHIBA plate was omitted.

All plates were incubated for at least 4 weeks at 28°C in air. Plates were examined daily during the first week, twice during the second week, and once a week subsequently. The day of inoculation was considered day 1; in this convention, cultures positive at, e.g., day 7 include all those which required more than 6 but no more than 7 full days to become positive. The day on which fungal growth was first noted (not the day of final identification) was recorded.

Frequency of positive cultures. Fungi were recovered from 24% (529 of 2,173) of cultures overall. The positive cultures derived from 331 patients (mean, 1.8; range, 0 to 20 positive cultures per patient). Most positive cultures (86%) were from inpatients, and about half (46%) were from patients who had been hospitalized for more than 1 week. The association between inpatient status and likelihood of a positive culture was statistically significant ($P < 0.001$, χ^2 test).

Respiratory specimens accounted for 45% of positives (237 of 529), stool specimens accounted for 24% (125 of 529), and urine specimens accounted for 11% (59 of 529) (Fig. 1). The highest rate of positivity occurred in stool specimens (of which 70% were positive), and the second highest rate occurred in respiratory specimens (45% positive).

A single fungal species was recovered in 86% (453 of 529) of positive cultures. Two species were grown from 69 cultures (13%), and three species were grown from 7 cultures (1.3%). In all, 612 distinct isolates were recovered (mean, 1.2 species per culture). Most of the specimens with multiple species derived from a respiratory source (Fig. 1).

Yeasts accounted for 79% (481 of 612), molds accounted for 20% (125 of 612), and dimorphic fungi accounted for less than 1% of isolates (6 of 612). The species isolated are listed in Table 1.

Time to detection. Overall, 94% (573 of 612) of all isolates had been detected by day 7 and more than 98% (601 of 612) had been detected by day 14. For urine, aspirate, tissue, and respiratory specimens, at least 95% of the isolates which were ultimately recovered had become evident by day 7 (Fig. 2). The rate of recovery at 7 days was slightly lower for cerebrospinal fluid (93%), stool (91%), and nose, mouth, and sinus (90%) specimens.

Of yeast isolates, 98% (469 of 481) were detected by day 7 and 99% (478 of 481) were detected by day 14, regardless of

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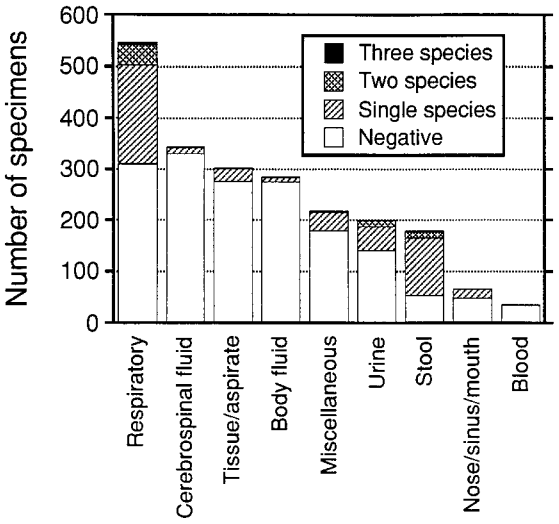

FIG. 1. Specimen types and proportions of positive and negative cultures.

site (Fig. 3). None of the three yeast isolates detected after 14 days were thought to be clinically significant. For molds, 81% (102 of 125) were detected by day 7 and 96% (120 of 125) were detected by day 14 (Fig. 3). Molds recovered after day 14 were only environmental contaminants of no clinical significance, e.g., a *Verticillium* sp. from sputum and a *Penicillium* sp. from stool. Detection of dimorphic fungi required more time: the mean time to detection was 14 days (median, 13 days; range, 4 to 33 days) (Fig. 3).

For cerebrospinal fluid specimens, all (14 of 14) isolates were detected by day 14. Nine of the 10 isolates of *Cryptococcus neoformans* were detected by day 5.

TABLE 1. Fungal species isolated

Species	*n*	% of total	No. of days to detection	
			Avg	Maximum
Yeasts (not *Cryptococcus* spp.; not identified further)	209	34.2	3	23
Candida albicans	191	31.2	3	14
Aspergillus spp.	64	10.5	5	21
Candida spp. (not *C. albicans*)	45	7.4	3	12
Penicillium spp.	25	4.1	6	23
Torulopsis glabrata	21	3.4	3	6
Cryptococcus neoformans	10	1.6	4	11
Blastomyces dermatitidis	5	0.8	16	33
Cladosporium spp.	5	0.8	7	14
Fusarium spp.	4	0.7	4	5
Paecilomyces spp.	4	0.7	9	20
Trichosporon beigelii	4	0.7	3	4
Bipolaris spp.	3	0.5	4	4
Acremonium spp.	2	0.3	4	4
Cunninghamella spp.	2	0.3	4	4
Fonsecaea pedrosoi	2	0.3	5	6
Rhodotorula spp.	2	0.3	5	5
Coccidioides immitis	1	0.1	4	4
Others[a]	13	2.6	8	2–22
Total	612	100.0	3.7	33

[a] Others included single isolates of *Alternaria*, *Aureobasidium*, *Blastoschizomyces capitatus*, *Chrysosporium*, *Cryptococcus albidus*, *Curvularia*, *Geotrichum*, *Malbranchea*, *Pseudallescheria boydii*, *Rhizopus*, *Saccharomyces cerevisiae*, *Verticillium*, and an unidentified mold.

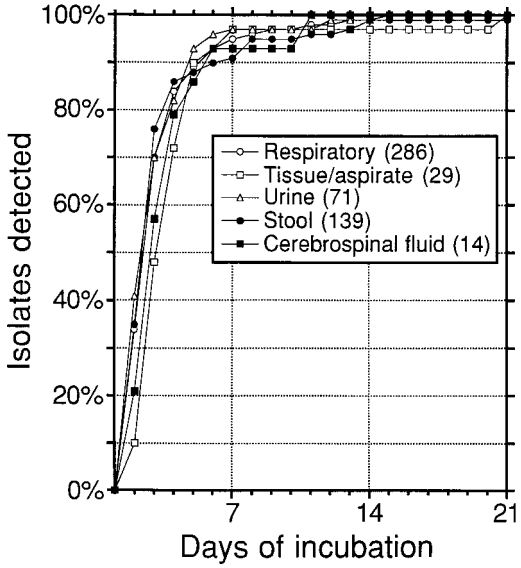

FIG. 2. Time to detection of fungal growth from various specimen types.

Detection of fungal growth occurred on average 1 day sooner for inpatients than for outpatients (3.6 days versus 4.6 days; $P < 0.05$, Student's t test). Length of inpatient stay was not associated with rapidity of detection.

Discussion. Traditional recommendations for the length of incubation of fungal cultures are based on the slow growth of some fungal species, notably dimorphic fungi (3, 4). Our results indicate that with current culture methods, 95% of all fungal isolates are recovered within 14 days, and most subsequent isolates represent environmental contaminants. Infection with dimorphic fungi was uncommon in our population and had usually been suggested clinically. In laboratories serving areas of high endemicity for dimorphic fungi, especially where human immunodeficiency virus infection is also common, it may be advisable to keep certain specimens for 4 weeks

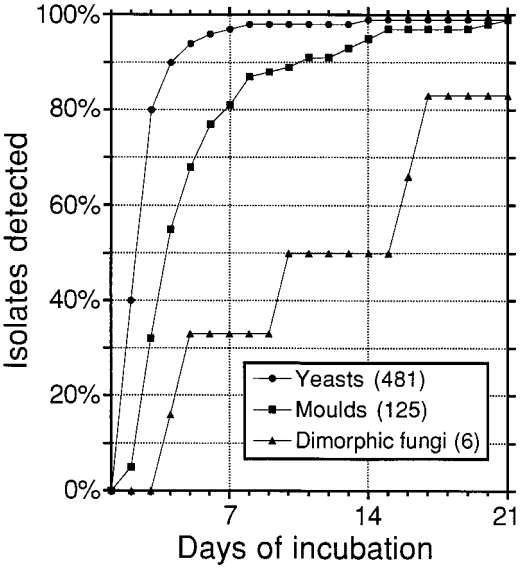

FIG. 3. Time to detection of fungal growth for yeasts, molds, and dimorphic fungi.

or more even when clinical suspicion of such an infection is low.

On the basis of these results, our laboratory now finalizes most cultures submitted for confirmation of yeast infection (e.g., stool, mouth, throat, and vaginal specimens) after a 1-week incubation. Urine specimens are held for 2 weeks. When molds are suspected, as from respiratory sites or tissues, we incubate cultures for 4 weeks, as we did in the past. With further data, it may be possible to reduce the routine incubation period for even these specimens.

REFERENCES

1. **Koneman, E. W., S. D. Allen, W. M. Janda, P. C. Schreckenberger, and W. C. Winn, Jr.** 1992. Color atlas and textbook of diagnostic microbiology, 34th ed., p. 805. J. B. Lippincott Company, Philadelphia.
2. **Kwon-Chung, K. J., and J. E. Bennett.** 1992. Medical mycology, p. 51. Lea & Febiger, Philadelphia.
3. **Larone, D. H.** 1995. Medically important fungi: a guide to identification, 3rd ed., p. 215. American Society for Microbiology, Washington, D.C.
4. **Merz, W. G., and G. D. Roberts.** 1995. Detection and recovery of fungi from clinical specimens, p. 709–722. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. M. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.