JCM
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Optimal Media for Use in Air Sampling To Detect Cultivable Bacteria and Fungi in the Pharmacy

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Current guidelines for air sampling for bacteria and fungi in compounding pharmacies require the use of a medium for each type of organism. U.S. Pharmacopeia (USP) chapter <797> (http://www.pbm.va.gov/linksotherresources/docs/USP797PharmaceuticalCompoundingSterileCompounding.pdf) calls for tryptic soy agar with polysorbate and lecithin (TSApl) for bacteria and malt extract agar (MEA) for fungi. In contrast, the Controlled Environment Testing Association (CETA), the professional organization for individuals who certify hoods and clean rooms, states in its 2012 certification application guide (http://www.cetainternational.org/reference/CAG-009v3.pdf?sid=1267) that a single-plate method is acceptable, implying that it is not always necessary to use an additional medium specifically for fungi. In this study, we reviewed 5.5 years of data from our laboratory to determine the utility of TSApl versus yeast malt extract agar (YMEA) for the isolation of fungi. Our findings, from 2,073 air samples obtained from compounding pharmacies, demonstrated that the YMEA yielded >2.5 times more fungal isolates than TSApl.

Compounded sterile preparations (CSPs) are pharmaceutical preparations that must be carefully and aseptically admixed by a licensed pharmacist or pharmacy tech in a specially designed and designated clean-room environment. This preparation is then administered to a patient, often by an intravenous (i.v.), intrathecal, or intraocular route (American Society of Health-System Pharmacists U.S. Pharmacopeia [USP] chapter <797> discussion guide for compounding sterile preparations, http://www.ashp.org/s_ashp/docs/files/discguide797-2008.pdf). Contaminated CSPs, such as total parenteral nutrition (TPN) and chemotherapy drugs made in hospital pharmacies and/or freestanding compounding pharmacies, are a notable source of serious infections (1–16; see also the CDC report of a multistate fungal meningitis outbreak [http://www.cdc.gov/hai/outbreaks/meningitis-map.html] and the FDA report of an intravenous compounded products recall [http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm249099.htm]). The USP created chapter <797> to establish standardized protocols to ensure the preparation of sterile and safe CSPs (17). A cornerstone of chapter <797> is the verification that the pharmacy clean-room environment is maintained free from significant sources of contamination (18). One of the approaches used for this process is air sampling to detect excessive bacterial and fungal organisms in the clean-room environment. USP chapter <797> specifies that air sampling be conducted semiannually at a minimum for both bacteria and fungi (see the American Society of Health-System Pharmacists USP chapter <797> discussion guide [http://www.ashp.org/s_ashp/docs/files/discguide797-2008.pdf]).

It is important to note that U.S. sterile i.v. compounding pharmacies operate under state licensing guidelines, and USP chapter <797> has not been universally adopted, so that actual practices vary across the country. Certified registered clean-room professionals (Certifiers), through their trade association guidelines, the Controlled Environment Testing Association (CETA) 2012 certification and application guide (see http://www.cetainternational.org/reference/CAG-009v3.pdf?sid=1267), describe two sampling methods, a single-plate method and a duplicate-plate method, thus implying that it is not always necessary to use an additional medium specifically for fungi. In order to determine whether bacterial and fungal media are both necessary, we did a retrospective look back and examined data from a 5.5-year time period of air sampling in 30 hospital pharmacies and two freestanding sterile compounding pharmacies. The environmental monitoring in these pharmacies was performed in the normal course of business by Microbiology Specialists Incorporated (MSI).

(R.A.J. presented a poster of this work at the Texas Tech Health Sciences Center–Paul L. Foster School of Medicine in fulfillment of a requirement to do clinical research in the Scholarly Activity and Research Program.)

MATERIALS AND METHODS

Air sampling was performed using a volumetric air sampler (SAS 180; Bioscience International, Rockville, MD). A bacterial medium (tryptic soy agar [TSA] with polysorbate and lecithin [TSApl]) and a fungal medium (yeast malt extract agar [YMEA]) were used. Both bacterial and fungal media were prepared and packaged by Anaerobe Systems, Morgan Hill, CA. Both media were packaged in sealed foil pouches with sterile absorbent pads; this prevents the accumulation of moisture (condensate) often seen with plates in cellophane sleeves when they are transported. Per USP chapter <797> instructions, a prede-

Received 9 April 2013 Returned for modification 6 May 2013
Accepted 29 July 2013
Published ahead of print 31 July 2013
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doi:10.1128/JCM.00944-13

TABLE 1 Total numbers of samples and failed samples per year

Yr	Total no. of samples	No. of failed samples	% of failed samples
2007	227	82	36
2008	306	81	26
2009	437	131	30
2010	563	204	36
2011	457	180	39
2012[a]	83	30	36

[a] Six months only.

termined volume of air (between 400 and 1,000 liters) was collected based on the size of the hoods, their location in the compounding areas, and the positioning of supply and return vents in each sterile compounding area.

One TSApl plate and one YMEA plate were collected at each location. Plates were all returned to MSI for analysis. The TSApl plates were incubated for 72 h at 35°C ± 2°C, and YMEA was incubated for 5 to 7 days at 28°C ± 2°C. A certified microbiologist then counted and identified the colonies on each plate. Results were reported as the number of CFU per cubic meter of air (CFU/m^3). Any potential pathogens were noted. USP chapter <797> defines action limits for hoods, i.v./buffer areas, and anterooms (where donning of personal protective equipment [PPE] occurs). In addition, the presence of a potential pathogen in any sample is considered significant, indicates that the area has failed to comply with action limits, and must be reported to the pharmacy immediately.

RESULTS

A total of 2,073 environmental samples for monitoring (air samples) were collected from compounding pharmacies and examined at Microbiology Specialists Incorporated (MSI).

Table 1 shows the total number of air samples collected per year and the number of sampling results that failed. Overall, in 5.5 years of testing, 34% of all air samples exceeded action limits or contained potential pathogens.

Table 2 shows the number of air samples collected per year and the number of sample results that contained potentially pathogenic bacteria. *Bacillus* spp. were the most frequently isolated bacterial contaminants. However, Gram-negative rods, such as *Enterobacteriaceae*, *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter*, and even *Mycobacterium* and *Nocardia* spp., were recovered.

Table 3 shows the number of air samples collected per year and the number of those sample results that contained potentially pathogenic fungi. *Aspergillus*, *Acremonium*, and *Fusarium* were the most frequently isolated molds, with a diverse group of other molds and yeasts also recovered.

Table 4 shows the numbers of potentially pathogenic bacteria and fungi side by side. Of note, during 4 of the 6 years, more potentially pathogenic fungi were isolated than potentially pathogenic bacteria.

Table 5 shows the total percentage of fungi that grew on YMEA versus TSApl. In fact, over the 5.5-year period, 285/388 (73%) of the fungi grew only on YMEA. The number of fungi that grew on TSApl alone was 103/388 (27%).

DISCUSSION

In 2004, the U.S. Pharmacopeia (USP) published chapter <797>, in which it outlined a program to ensure that sterile compounded preparations were indeed sterile when they reached the patient (17). The chapter was revised in 2007 (see http://www.pbm.va.gov/linksotherresources/docs/USP797PharmaceuticalCompoundingSterileCompounding.pdf). From the microbiology perspective, the program involves environmental monitoring in the i.v. pharmacy, as well as a robust program to validate compounder competency (19). During this time, there were already incidences of patient morbidity and mortality from contaminated preparations, thus demonstrating a need for such a program (3, 5, 6, 7, 10, 12, 13, 14, 15, 16). Using microbial fingerprinting, we have previously shown that the same *Brevibacillus brevis* found in the air of a pharmacy clean room was also isolated from an i.v. bag prepared under the hood in the clean room that same day (19).

Unfortunately, there is currently no mandatory accreditation or inspection for pharmacies. Furthermore, many state boards of pharmacy have not even adopted the chapter <797> rules. Perhaps, as professionals and organizations who are involved with CSP preparation see an environmental air sample test failure rate similar to the ones described here, which average 33% per year over the 5.5-year period analyzed, the compounders will embrace a robust quality assurance program which will mitigate contamination in the pharmacy. Our results indicate that this type of monitoring should include a specific fungal medium to reliably demonstrate the presence of fungal contaminants, which may also be human pathogens.

On 31 January 2012, the CETA published its certification application guide detailing its position on USP chapter <797> viable environmental sampling (http://www.cetainternational.org/reference/CAG-009v3.pdf?sid=1267). In it, CETA advocates the use of a general microbiological medium such as tryptic soy agar, its so-called single-plate method. CETA advocates

TABLE 2 Samples with potentially pathogenic bacteria

Yr	Total no. of samples	No. of *Bacillus* spp.	No. of Gram-negative rods	No. of aerobic *Actinomycetes*	No. of samples with other bacteria
2007	227	46	6		
2008	306	33	4		3 (1 *Serratia*, 1 *Pseudomonas*, 1 *Stenotrophomonas*)
2009	437	78	25	9	16 (6 *Pseudomonas*, 3 *Stenotrophomonas*, 2 *Mycobacterium*, 2 *Sphingomonas*, 1 *Enterobacter*, 1 *Acinetobacter*, 1 *Brevundimonas*)
2010	563	100	57	28	3 (*Acinetobacter*)
2011	457	79	33	31	4 (1 *Sphingomonas*, 3 *Acinetobacter*)
2012[a]	83	19	9	1	

[a] Six months only.

TABLE 3 Samples with potentially pathogenic fungi

Yr	Total no. of samples	No. of samples with:				No. of samples with other fungi
		Aspergillus	*Acremonium*	*Fusarium*	Yeast	
2007	227	19	2	7	3	14 (3 *Aureobasidium*, 2 *Hormographiella*, 4 *Rhizopus*, 3 *Pithomyces*, 1 *Sporothrix*, 1 *Ochroconis*)
2008	306	17	3	2	3	13 (1 *Scopulariopsis*, 3 *Paecilomyces*, 2 *Aureobasidium*, 6 *Pithomyces*, 1 *Microsporum*)
2009	437	17	5	4	8	16 (1 *Sporothrix*, 3 *Aureobasidium*, 4 *Scopulariopsis*, 1 *Tritarachium roseum*, 1 *Cunninghamella*, 1 *Verticillium*, 4 *Paecilomyces*, 1 *Rhizopus*)
2010	563	34	13	13	30	17 (2 *Geotrichum*, 2 *Paecilomyces*, 2 *Sporothrix*, 1 *Hormographiella*, 1 *Ochroconis*, 3 *Microsporum*, 1 *Hyalodendron*, 1 *Beauveria*, 1 *Rhinocladiella*, 3 *Pithomyces*)
2011	457	39	18	10	19	12 (1 *Scopulariopsis*, 1 *Paecilomyces*, 3 *Malbranchea*, 1 *Trichoderma*, 4 *Geotrichum*, 1 *Basipetospora rubra*, 1 *Zygomycetes*)
2012[a]	83	1	0	0	7	1 (*Aureobasidium*)

[a] Six months only.

incubating these plates at 30°C to 35°C for 48 to 72 h and then reincubating the same plates at 26°C to 30°C for 5 to 7 days. What CETA is advocating does not match any published standard method for examination of bacteria or fungi that we know of. In fact, USP chapter <797> calls for TSA to be incubated at 30°C to 35°C for 48 to 72 h and malt extract agar or other suitable fungal media to be incubated at 26°C to 30°C for 5 to 7 days.

Our data do not support the CETA approach. Ideally, a study should be set up comparing the USP and CETA methods. However, we worry that lowering the temperature after incubation at 35°C, as stated in the CETA guideline, would cause the inhibition of fungal growth and inability to sporulate. Further, having to perform additional subcultures because of inhibited or sterile growth would mean that getting information to the i.v. compounding pharmacy would subsequently be delayed. We were able to do tape preparations or tease mounts to identify the fungi directly from YMEA. The idea here is that the use of fungal media when looking for fungi is a primary tenet of microbiology. We question why hood and clean-room certifiers are even discussing best practices for the microbiology laboratory in the CETA document, especially when advocating a method not even approved in USP chapter <797>. In any case, analysis of 5.5 years of data of 2,073 environmental air samples has shown that two media (one bacterial and one fungal) must be used in order to effectively track and correct any problems.

TABLE 4 Total percentages of potentially pathogenic bacteria and fungi

Yr	Potentially pathogenic bacteria		Potentially pathogenic fungi	
	Total no.	% of failed samples	Total no.	% of failed samples
2007	33	40	49	60
2008	36	44	45	56
2009	76	58	55	42
2010	89	44	115	56
2011	67	37	113	63
2012[a]	19	63	11	37

[a] Six months only.

TABLE 5 Total percentages of fungi on TSApl and YMEA

Yr	Total no. of samples	Fungi on YMEA		Fungi on TSApl	
		Total no.	%	Total no.	%
2007	49	39	80	10	20
2008	45	34	76	11	24
2009	55	39	71	16	29
2010	115	86	75	29	25
2011	113	79	70	34	30
2012[a]	11	8	73	3	27
Total	388	285	73	103	27

[a] Six months only.

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