

Side by Side Comparison of the Aerotrap Total Fungal SamplerTM with Grease Slides, the Aerotrap Total Fungal SamplerTM with Glyceryl Triacetate Cleared Mixed Cellulose Ester Membrane Filter Slides and the Zefon Bio-Pump^R using the Zefon Air-O-Cell^R.

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Purpose:

To examine the collection efficiency of three different types of adhesive collection media used in non-viable spore trap sampling.

Introduction:

Non-viable spore trap samples are among the most common types of environmental samples taken for the purpose of assessing the concentration of fungal spores in the air. Many of the common samplers are based on the same principal. A known volume of air is pulled through a small opening and the particulates in the air stream impact on a surface coated with an adhesive medium. The shape of the opening dictates the shape of the impaction area, known as the trace. The samplers used in this survey lay down a trace in a straight line. The Zefon Bio-Pump^R was one of the samplers used in this survey. The Zefon Air-O-Cell^R is used in conjunction with the Zefon Bio-Pump^R and uses an acrylic based adhesive medium inside a cassette that fits onto the sampler. The media within the cassette is never exposed to the environment before sampling. Other samplers have taken a different approach. The Aerotrap Total Fungal SamplerTM, which will be referred to hereafter as the Aerotrap has the adhesive media applied directly to a standard microscope slide which is then placed inside the sampler. The adhesive media typically used in conjunction with this type of sampler are grease of some sort or mixed cellulose ester filters (MCE) cleared with a solvent which chemically alters the filter making it both adhesive and transparent. This survey compared the Zefon Bio-Pump^R sampler using Zefon Air-O-Cells^R, the Aerotrap using grease as a collection media and the Aerotrap using glyceryl triacetate cleared mixed cellulose ester membrane filters (MCE) as a collection media. In this comparison survey, samples were run side by side at the same location for the same period of time. Samples were then analyzed and the data collected to determine if there are any differences in collection efficiency between the samplers and the adhesive media used.

Materials and Methods:

The Collection Media

The grease slides were prepared by applying a thin layer of Thomas Scientific stopcock grease to standard 75 X 25 X 1-1.2 mm microscope slides.

The cleared Mixed Cellulose Ester Membrane Filter Slides were prepared by submerging 25mm 0.45 um pore size Millipore Mixed Cellulose Ester (MCE) membrane filters in > 99 % glyceryl triacetate solution, wiping away the excess liquid and then applying them to the center of a standard 75 X 25 X 1-1.2 mm microscope slide. The filters clear within an hour and were allowed to dry for 24 hours before use.

Zefon Air-O-Cell^R's require no preparation.

The Samplers

The Aerotrap was used to take samples with the grease and cleared MCE samples.

The Zefon Bio-Pump^R was used to take the Zefon Air-O-Cell^R samples.

Sampling protocol

Samplers were calibrated to 15L/min at every new location or every 5th round of sampling. Samplers were placed 1 meter from one another and run simultaneously at 15L/min for 5 minutes for a total of 75 liters.

100 rounds of 3 samples were taken for a total of 300 samples. Each round had 1 Aerotrap with grease as an adhesive medium sample, 1 Aerotrap with cleared MCE as an adhesive medium and 1 Zefon Air-O-Cell^R sample.

- 100 using the Aerotrap with grease
- 100 using the Aerotrap with cleared MCE
- 100 using the Zefon Air-O-Cell^R

Examination of samples

Calberla's solution was used as a mounting fluid to prepare the grease slides for microscopic examination.

Lato-Phenol Cotton Blue in lactic acid was used as a mounting fluid for the cleared MCE slides and Zefon Air-O-Cell^R samples.

Samples were then examined microscopically at 400X.

**Average Spores Recovered Per Sample Over Entire Survey
(Total Sample Volume 7500L)
Most Recovered Spore Types Only**

Genera	Grease	Cleared MCE	Zefon
Alternaria	1.41	1.00	0.77
Ascospores	6.29	4.91	4.74
Basidiospores	40.27	65.06	84.71
Cladosporium	95.87	116.7	108.0
Penicillium / Aspergillus type	10.36	13.43	10.26
Smut, Periconia, Myxomycetes group	17.19	14.27	5.55
Unknown Brown	7.65	6.27	5.27
Hyphal Elements	10.98	7.88	6.22
Sum	190.0	229.5	225.5

Summary of Data for Total Counts

	Grease	Cleared MCE	Zefon
Total Spores Recovered All Genera All Spore Types (Volume 7500L)	20029	24622	24555
Total spores counted	69206		
Percent of Total Spores Recovered using Grease	28.94		
Percent of Total Spores Recovered using Cleared MCE	35.57		
Percent of Total Spores Recovered using Zefons	35.48		
Percentage of rounds in which the total count on the grease slide exceeded that of the Cleared MCE slide	29.34		
Percentage of rounds in which the total count on the Cleared MCE slide exceeded that of the Grease slide	70.65		
Percentage of rounds in which the total count on the Grease slide exceeded that of the Zefon slide	30.43		
Percentage of rounds in which the total count on the Zefon slide exceeded that of the Grease slide	69.57		
Percentage of rounds in which the total count on the Zefon slide exceeded that of the Cleared MCE slide	37.36		
Percentage of rounds in which the total count on the Cleared MCE slide exceeded that of the Zefon slide	62.64		

Summary of Data For individual Genera

Alternaria	Grease	Cleared MCE	Zefon
Totals	141	100	77
Percentage of rounds in which the Alternaria count on the grease slide exceeded that of the Cleared MCE slide	65.63		
Percentage of rounds in which the Alternaria count on the Cleared MCE slide exceeded that of the Grease slide	34.38		
Percentage of rounds in which the Alternaria count on the Grease slide exceeded that of the Zefon slide	59.38		
Percentage of rounds in which the Alternaria count on the Zefon slide exceeded that of the Grease slide	40.63		
Percentage of rounds in which the Alternaria count on the Zefon slide exceeded that of the Cleared MCE slide	46.88		
Percentage of rounds in which the Alternaria count on the Cleared MCE slide exceeded that of the Zefon slide	53.13		
Ascospores	Grease	Cleared MCE	Zefon
Totals	629	491	474
Percentage of rounds in which the Ascospores count on the grease slide exceeded that of the Cleared MCE slide	68.89		
Percentage of rounds in which the Ascospores count on the Cleared MCE slide exceeded that of the Grease slide	31.11		
Percentage of rounds in which the Ascospores count on the Grease slide exceeded that of the Zefon slide	65.56		
Percentage of rounds in which the Ascospores count on the Zefon slide exceeded that of the Grease slide	34.45		
Percentage of rounds in which the Ascospores count on the Zefon slide exceeded that of the Cleared MCE slide	40.00		
Percentage of rounds in which the Ascospores count on the Cleared MCE slide exceeded that of the Zefon slide	60.00		

Basidiospores	Grease	Cleared MCE	Zefon
Totals	4027	6506	8471
Percentage of rounds in which the Basidiospores count on the grease slide exceeded that of the Cleared MCE slide	20.88		
Percentage of rounds in which the Basidiospores count on the Cleared MCE slide exceeded that of the Grease slide	79.12		
Percentage of rounds in which the Basidiospores count on the Grease slide exceeded that of the Zefon slide	26.37		
Percentage of rounds in which the Basidiospores count on the Zefon slide exceeded that of the Grease slide	73.63		
Percentage of rounds in which the Basidiospores count on the Zefon slide exceeded that of the Cleared MCE slide	51.65		
Percentage of rounds in which the Basidiospores count on the Cleared MCE slide exceeded that of the Zefon slide	48.35		
Cladosporium			
Totals	9587	11670	10802
Percentage of rounds in which the Cladosporium count on the grease slide exceeded that of the Cleared MCE slide	43.48		
Percentage of rounds in which the Cladosporium count on the Cleared MCE slide exceeded that of the Grease slide	56.52		
Percentage of rounds in which the Cladosporium count on the Grease slide exceeded that of the Zefon slide	54.95		
Percentage of rounds in which the Cladosporium count on the Zefon slide exceeded that of the Grease slide	45.06		
Percentage of rounds in which the Ascospores count on the Zefon slide exceeded that of the Cleared MCE slide	47.83		
Percentage of rounds in which the Cladosporium count on the Cleared MCE slide exceeded that of the Zefon slide	52.17		

Penicillium / Aspergillus group	Grease	Cleared MCE	Zefon
Totals	1036	1343	1026
Percentage of rounds in which the Penicillium / Aspergillus count on the grease slide exceeded that of the Cleared MCE slide	36.25		
Percentage of rounds in which the Penicillium / Aspergillus count on the Cleared MCE slide exceeded that of the Grease slide	63.75		
Percentage of rounds in which the Penicillium / Aspergillus count on the Grease slide exceeded that of the Zefon slide	51.25		
Percentage of rounds in which the Penicillium / Aspergillus count on the Zefon slide exceeded that of the Grease slide	48.75		
Percentage of rounds in which the Penicillium / Aspergillus count on the Zefon slide exceeded that of the Cleared MCE slide	33.75		
Percentage of rounds in which the Penicillium / Aspergillus count on the Cleared MCE slide exceeded that of the Zefon slide	66.25		
Smut, Periconia, Myxomycetes group	Grease	Cleared MCE	Zefon
Totals	1719	1427	555
Percentage of rounds in which the Smut, Periconia, Myxomycetes group count on the grease slide exceeded that of the Cleared MCE slide	60.00		
Percentage of rounds in which the Smut, Periconia, Myxomycetes group count on the Cleared MCE slide exceeded that of the Grease slide	40.00		
Percentage of rounds in which the Smut, Periconia, Myxomycetes group count on the Grease slide exceeded that of the Zefon slide	74.67		
Percentage of rounds in which the Smut, Periconia, Myxomycetes group count on the Zefon slide exceeded that of the Grease slide	25.33		
Percentage of rounds in which the Smut, Periconia, Myxomycetes group count on the Zefon slide exceeded that of the Cleared MCE slide	30.67		
Percentage of rounds in which the Smut, Periconia, Myxomycetes group count on the Cleared MCE slide exceeded that of the Zefon slide	69.33		

	Grease	Cleared MCE	Zefon
Unknown, Brown			
Totals	765	627	527
Percentage of rounds in which the Unknown, Brown count on the grease slide exceeded that of the Cleared MCE slide	64.29		
Percentage of rounds in which the Unknown, Brown count on the Cleared MCE slide exceeded that of the Grease slide	35.71		
Percentage of rounds in which the Unknown, Brown count on the Grease slide exceeded that of the Zefon slide	61.43		
Percentage of rounds in which the Unknown, Brown count on the Zefon slide exceeded that of the Grease slide	38.57		
Percentage of rounds in which the Unknown, Brown count on the Zefon slide exceeded that of the Cleared MCE slide	47.14		
Percentage of rounds in which the Unknown, Brown count on the Cleared MCE slide exceeded that of the Zefon slide	52.86		
Average Percentage of Trace readable at 14 days (all samples)			
Cleared MCE	100		
Grease	47.78		
Zefon	100		

Discussion:

The Data

The total counts of the cleared MCE and Zefon Air-O-Cell^R samples were 24622 and 24555 respectively. The total count for grease slides was 20029. The total count on cleared MCE slides exceeded that of grease slides in 70.65% of the rounds conducted. The total count on Zefon Air-O-Cell^R slides exceeded that of grease in 69.57% of the rounds conducted. The total count on cleared MCE slides exceeded that of Zefon Air-O-Cell^R slides in 62.64% of the rounds conducted. Cleared MCE slides had the highest recovery rate of total spores for the majority of rounds against either grease slides or Zefon Air-O-Cell^R.

The *Alternaria* count of the cleared MCE and Zefon Air-O-Cell^R were 100 and 77 respectively. The *Alternaria* count for grease slides was 141. The *Alternaria* count on cleared MCE slides exceeded that of grease slides in 34.38% of the rounds conducted. The *Alternaria* count on Zefon Air-O-Cell^R slides exceeded that of grease in 40.63% of the rounds conducted. The *Alternaria* count on cleared MCE slides exceeded that of Zefon Air-O-Cell^R slides in 53.13% of the rounds conducted. The grease slides had the highest recovery rate of *Alternaria* for the majority of rounds conducted. The average *Alternaria* count over the entire survey was: grease 1.41, cleared MCE 1.0 and Zefon Air-O-Cell^R 0.77.

The ascospore count of the cleared MCE and Zefon Air-O-Cell^R were 491 and 474 respectively. The ascospore count for grease slides was 629. The ascospore count on cleared MCE slides exceeded that of grease slides in 31.11% of the rounds conducted. The ascospore count on Zefon Air-O-Cell^R slides exceeded that of grease in 34.45% of the rounds conducted. The ascospore count on cleared MCE slides exceeded that of Zefon Air-O-Cell^R slides in 60.00% of the rounds conducted. The grease slides had the highest recovery rate of ascospore for the majority of rounds conducted. The average ascospore count over the entire survey was: grease 6.29, cleared MCE 4.91 and Zefon Air-O-Cell^R 4.74.

The basidiospore count of the cleared MCE and Zefon Air-O-Cell^R were 6506 and 8471 respectively. The basidiospore count for grease slides was 4027. The basidiospore count on cleared MCE slides exceeded that of grease slides in 79.12% of the rounds conducted. The basidiospore count on Zefon Air-O-Cell^R slides exceeded that of grease in 73.63% of the rounds conducted. The basidiospore count on cleared MCE slides exceeded that of Zefon Air-O-Cell^R slides in 48.35% of the rounds conducted. The Zefon Air-O-Cell^R slides had the highest recovery rate of basidiospore for the majority of rounds conducted. The average basidiospore count over the entire survey was: grease 40.27, cleared MCE 65.06 and Zefon Air-O-Cell^R 84.71.

The *Cladosporium* count of the cleared MCE and Zefon Air-O-Cell^R were 11670 and 10802 respectively. The *Cladosporium* count for grease slides was 9587. The *Cladosporium* count on cleared MCE slides exceeded that of grease slides in 56.52% of the rounds conducted. The *Cladosporium* count on Zefon Air-O-Cell^R slides exceeded that of grease in 45.06% of the rounds conducted. The *Cladosporium* count on cleared MCE slides exceeded that of Zefon Air-O-Cell^R slides in 52.17% of the rounds conducted. The cleared MCE slides had the highest recovery rate of *Cladosporium* for the majority of rounds conducted. The average *Cladosporium* count over the entire survey was: grease 95.87, cleared MCE 116.70 and Zefon Air-O-Cell^R 108.00.

The *Penicillium* / *Aspergillus* type count of the cleared MCE and Zefon Air-O-Cell^R were 1343 and 1026 respectively. The *Penicillium* / *Aspergillus* count for grease slides was 1036. The *Penicillium* / *Aspergillus* type count on cleared MCE slides exceeded that of grease slides in 63.75% of the rounds conducted. The *Penicillium* / *Aspergillus* type count on Zefon Air-O-Cell^R slides exceeded that of grease in 48.75% of the rounds

conducted. The *Penicillium / Aspergillus* type count on cleared MCE slides exceeded that of Zefon Air-O-Cell^R slides in 66.25% of the rounds conducted. The Cleared MCE slides had the highest recovery rate of *Penicillium / Aspergillus* type for the majority of rounds conducted. The average *Penicillium / Aspergillus* type count over the entire survey was: grease 10.36, cleared MCE 13.43 and Zefon Air-O-Cell^R 10.26.

The Smut, *Periconia*, Myxomycetes group count of the cleared MCE and Zefon Air-O-Cell^R were 1427 and 555 respectively. The Smut, *Periconia*, Myxomycetes count for grease slides was 1719. The Smut, *Periconia*, Myxomycetes group count on cleared MCE slides exceeded that of grease slides in 40.00% of the rounds conducted. The Smut, *Periconia*, Myxomycetes group count on Zefon Air-O-Cell^R slides exceeded that of grease in 25.33% of the rounds conducted. The Smut, *Periconia*, Myxomycetes group count on cleared MCE slides exceeded that of Zefon Air-O-Cell^R slides in 69.33% of the rounds conducted. The grease slides had the highest recovery rate of Smut, *Periconia*, Myxomycetes group for the majority of rounds conducted. The average Smut, *Periconia*, Myxomycetes group count over the entire survey was: grease 17.19, cleared MCE 14.27 and Zefon Air-O-Cell^R 5.55.

The Unknown Brown count of the cleared MCE and Zefon Air-O-Cell^R were 627 and 527 respectively. The Unknown Brown count for grease slides was 765. The Unknown Brown count on cleared MCE slides exceeded that of grease slides in 35.71% of the rounds conducted. The Unknown Brown count on Zefon Air-O-Cell^R slides exceeded that of grease in 38.57% of the rounds conducted. The Unknown Brown count on cleared MCE slides exceeded that of Zefon Air-O-Cell^R slides in 52.86% of the rounds conducted. The grease slides had the highest recovery rate of Unknown Brown for the majority of rounds conducted. The average Unknown Brown count over the entire survey was: grease 7.65, cleared MCE 6.27 and Zefon Air-O-Cell^R 5.27.

The Advantages and Disadvantages of the collection media

Each sampler and adhesive media has its advantages and disadvantages. Zefon Air-O-Cell^R's are expensive when compared with grease slides or cleared MCE slides. Zefon Air-O-Cell^R's also require a setup time. The small adhesive slide within the cassette must be removed and mounted on a standard microscope slide. Grease slides are inexpensive to make but the grease often separates and causes severe distortions of the trace. Cleared MCE slides are more costly than grease but the advantage is that they do not separate and when used in conjunction with lactic acid as a mounting media, the traces can be readable for years. Grease slides can deteriorate very quickly, often while the analyst is examining the slide. After 14 days all the Zefon Air-O-Cell^R's and cleared MCE slides were readable. The readability of the grease slides on average at this time was only about 48%. That means that on average only 48% of the trace was readable at 14 days. Grease slides are also more sensitive to disruption and are easily smudged in the field. Smudges cause problems in the laboratory. They upset the fine layer of grease and running occurs almost immediately. The data suggests all samplers and media studied can be used without any fear of detrimental consequences, but the grease is riskier due to its propensity to run.

Variability of Ambient Air Sampling and other considerations

Any type of ambient air sampling is inherently variable. Air currents are ever-changing and concentrations of particulates can vary greatly. Judgment must always be used when interpreting spore trap data. Variation in spore concentrations between samples in the same round in this survey commonly exceeded 50%. Spore trap samples are read by human beings and the accuracy of the sample depends on their skill at distinguishing spores from other types of biological and non-biological particles. Recognizing the same spore types on the different types of adhesive media can be difficult and requires practice. Human error plays a real factor in the accuracy of spore trap data. Despite the inherent variation in air sampling and the fallibility of the human analyst, the data from this survey indicates that Zefon Air-O-Cell^R's and cleared MCE may in the long run recover more spores than grease. The only way to determine if the general trends observed in the data above are accurate is to do controlled chamber studies, where the atmosphere being sampled had a known concentration of particles.

Comments or questions related to this survey should be directed to

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