CYCLEX ANALYSIS
STANDARD OPERATING PROCEDURE
FOR EXAMINING 10% OF TOTAL TRACE

Slide Preparation

Cyclex slide mailer should only be opened in the laboratory (or clean room conditions). The mailer sealing band should be cut, and the glass slide (containing the sample circle area) removed. No staining media is necessary; however conditions may dictate the use of certain stains. Cover the trace area with a cover slip (put the cover slip on at an angle). Label the slide with the sample number on the top and the batch number on the bottom of the slide with a sharpie marker.

Microscopic Examination Theory

Analysis of the collected sample should be performed by an experienced Microbiologist, Aerobiologist, or Environmental Microscopist. Counting and quantification of sample components is conducted by counting calibrated cross-sections of the deposited sample trace. The number and type of particles counted per cubic meter of air is calculated based on the diameter of the deposition trace, area of trace actually examined, volume of air collected, and number of particles counted.

Recommended Microscopic Counting Guidelines

10% of the trace should be examined. Identification and speciation should be performed at a minimum magnification of 400 - 600X

Overview

1. Kohler Illumination or Phase
2. Measure field diameters on microscope
3. Know flow rate and sample time
4. Know trace diameter (4.9 mm as specified by EMS)
5. Calculations

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Microscope

Make sure the microscope is daily aligned and logged in a calibration book. Currently, analysis is performed at 40X with 15X oculars, giving a total magnification of 600X.

The actual field diameter of each microscope is different and should be measured and recorded:
The measurements should be copied onto a sticker and placed on the microscope for reference.
Each microscope is different, and each different combination of ocular and objective lens must be calibrated separately.

(Field diameters below are estimated and are used for this example only –please measure each individual microscope and adjust the protocol accordingly)
15X Ocular 10X 1.43mm 40X 0.35mm 100X 0.13mm

Trace Measurement
The Cyclex-d cassette has a trace diameter of 4.9 mm.
2.45mm X 2.45mm X 3.141592 = 18.857405 (mm^2) total trace impact area
Circle Area = Pi x r^2

Calculation to Determine # of Fields to count 10% of the Cyclex-d sample trace w/ 40X Obj.
Total microscope field of view surface area = 0.175mm X 0.175mm X 3.141592 = 0.0962mm^2
Field of View Area = Pi x r^2
18.857405mm^2 / 0.0962mm^2 = 196.022 or 196 fields equals entire trace area
Total impact area / microscope field of view

# of fields required to analyze 10% of trace
196 X .10 = 19.6 OR 20 fields

Calculation to Determine Raw Count Multiplier
Trace Size mm^2 / Percentage Read mm^2 = Raw Count Multiplier
18.857405 mm^2 / 1.924 mm^2 = 9.8 OR 10

Calculation to Determine Multiplier for Air Volumes
1000L / Total air volume = *multiplier for air volume*

Final Calculation
# of spores counted (X) raw count multiplier (X) air volume multiplier = spores/M^3

Calculation to Determine Detection Limit
(1000L / Total Air Volume) / Percentage Read (expressed as a decimal point – example .25 for 25%)

Counting/Identification

Counting and identification of the various fungal types is the tricky part. Several reference books are available for use in the laboratory. If over 500 fungal spores are counted, then a note is to be added in the comment section on the report saying results are estimated.

Fungal counts are listed by each type of spore as indicated on the bench sheet. Lines are to be drawn to separate traverses. Use the 10X objective to find beginning, end, top, and bottom of the trace. Count with the 40X objective and use the 100X objective for clarification. Use of a phase contrast microscope, in general, makes for easier counting: take the phase out to make it brighter to see more overall, put the phase in to make it darker for more detail.

Background Debris

Background debris is an indication of overall particulate matter present in the air.
Use the following debris scale:

0 = no amount, no particles on slide (may indicate improper sampling)
1 = small amount, no affect on counts
2 = limited amount, counts may be underestimated
3 = large amount, counts underestimated
4 = overloaded, counts not available due to excessive debris