MICRO5 ANALYSIS
STANDARD OPERATING PROCEDURE FOR
EXAMINING 100% OF TOTAL TRACE

Slide Preparation

Micro5 cassettes should only be opened in the laboratory. The sealing band should be removed (or cut), and the glass cover slip (containing the sample trace, circle area) removed and slowly placed on a slide, media collection side up for 100x objective use (or if preferred sample can be prepped sample side down if 100x objective will not be used.). No staining media is necessary; however conditions may dictate the use of certain stains. 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

100% of the trace should be examined. Certain situations (i.e. overloads, heavy particulate) will dictate a smaller percentage be read (see other % reads for applicable protocol) 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 (2.3 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 Micro5 cassette has a trace length diameter of 2.3 mm.
1.15mm X 1.15mm X 3.141592 = 4.1547554 (mm^2) total trace impact area
Circle Area = Pi x r^2

Calculation to Determine # of Fields to count 100% of the Micro5 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
4.1547554mm^2 / 0.0962mm^2 = 43.1 or 43 fields equals entire trace area
Total impact area / microscope field of view

# of fields required to analyze 100% of trace
100% read = 43 fields w/ estimated 40x objective

Calculation to Determine Raw Count Multiplier
With 100% read multiplier will be 1

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