



Quantitative PCR Analysis: Basic Questions and Answers for Investigators

Q1: Is there a “hold time” from sample collection in the field to shipment and receipt into the laboratory?

YES! The time between sample collection and receipt into the laboratory for analysis should be within 24 hours. If a further delay is necessitated, samples should not arrive any later than 72 hours from collection. The minimization of time between sample collection and receipt into the laboratory ensures sample integrity and DNA preservation.

Q2: Which field sampling methods can be used for PCR analysis and how are samples collected?

Both air and dust samples can be collected for PCR analysis. Both require a polycarbonate or Teflon filter, 0.45 μm to 1.0 μm pore size. Air samples are collected using a sampling volume of approximately 3.5 liters/min for 3 to 10 hours. Most commonly, 8 hour sampling intervals are utilized. Areas with heavy background or dust should be sampled for shorter intervals, as these background materials may potentially clog the pores in sampling filters. Dust samples are collected using a high volume pump (15 liters/min), typically attached to a 37 mm cassette with a MCE filter and 1 inch length of tubing attached to the inlet. Dust samples are obtained by vacuuming a specified area of floor surface for 3 to 5 minutes. If hardwood floors are sampled, it may be necessary to vacuum the entire open floor area of the room for sufficient sample. The sampling volume (flow rate and hours sampled) must be indicated on the Chain of Custody with the submission of samples.

Q3: Where can I obtain sampling supplies?

Air and dust sampling cassettes and filters can be ordered directly from the laboratory. There is no additional cost for supplies ordered from the laboratory if the samples are then submitted for PCR analysis. Should you wish to order materials directly from the manufacturer, the following information may be useful:

Manufacturer: Zefon International, 1-800-282-0073

Air Samples: Product Description: 37 mm, 2-piece cassette, PC filter, 0.8 μm pore size, Product #728PC

Dust Samples: Product Description: 37mm, micro-vac cassette, 0.45 μm MCE filter, 1” tubing, Product #7345CC

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Q4: How quickly will I receive results from my samples?

Pure Earth Environmental Laboratory offers both 24 and 48 hour “turn-around” times for PCR samples. (Weekends not included)

Q5: How does PCR analysis compare to standard culture and spore trap methods?

PCR analysis cannot be directly compared to traditional methods such as culture and spore trap analysis for a variety of reasons. Due to its high sensitivity (often detecting a single spore), a PCR result may be orders of magnitude higher in organism concentration than a culture of the exact same sample. Factors such as out-competition by more aggressively growing molds or bacteria, specialized incubation and growth requirements for successful recovery of certain fungi in laboratory culture, qualitative and quantitative inaccuracies by laboratory personnel, among others, are commonly problematic when isolating and identifying fungi in culture. PCR analysis offers a standardized, reproducible methodology, for detecting and enumerating target organisms. Additionally, whereas traditional methods separately categorize “viable” organisms, as in culture samples, “viable” and potentially “non-viable” organisms, as in tape lifts and spore traps, PCR analysis equally represents both categories, as both groupings of organisms may be substantial in toxic or allergenic properties.

Q6: What are the advantages of using quantitative PCR analysis?

The advantages of PCR analysis are remarkable. Airborne fungal populations, cannot be reliably represented, nor can the determination of long term exposure be assessed, though small volume and short interval sampling, as is commonly practiced. PCR samples can be obtained over a time span of hours, yet remain unhindered by fungal overcrowding. PCR offers the highest sensitivity for detection and quantification of target organisms, even in small quantities. It is highly specific, unmistakably identifying molds to the species level. Samples can be easily collected with simple equipment and small quantities of material can be analyzed, with successful results. Results are readily available, within 24 or 48 hours. Additionally, PCR is a standardized, unbiased methodology, producing statistically verified results.

Q7: When should I use PCR analysis in my investigations?

PCR analysis should always be used when certain species of organisms are of concern. Definitive speciation and standardized quantification of fungal targets yields investigative results more valid, reproducible, and defensible. PCR should be utilized as a means of assessment for human exposure to fungal species over longer-time intervals, than are currently practiced. It is highly desirable when rapid turn-around time is needed for analytical results.

Q8: If PCR is species specific, why are multiple species reported for the same organism in my results?

Although the majority of fungal species can be definitively identified by DNA-based analysis, a few organisms share genetic similarities, undistinguishable by the current PCR technology. Organisms in these groups are also commonly similar in other properties as well, such as allergenicity and environmental niches.

Q9: Should I submit a field blank with my samples?

YES! Although positive and negative controls are performed in conjunction with every batch of samples in the laboratory, these measures are internal controls and only ensure sample integrity once the samples have entered the laboratory. A field blank is a valuable control measure, assessing the ENTIRE analytical process (from sample collection in the field to processing and data generation in the laboratory). A field blank is typically an unopened cassette containing the appropriate filter, and should accompany test samples from sample collection in the field to submission of samples to the laboratory. The field blank then proceeds through the sample analytical procedure, at the same time, as test samples. It verifies the integrity of the entire analytical process. It is at the discretion of the customer whether a field blank will be submitted.

Q10: How will I interpret my results?

Foremost, results of PCR analysis will document the occurrence of selected fungal species in air and dust samples. The presence of specific, quantifiable, fungal species will aid in guiding investigative and remedial strategies. Although no absolute guidelines currently exist in defining “normal” and “abnormal” or “alert” levels of fungal species by PCR analysis, an effective interpretative strategy may be to collect samples in “complaint” and “non-complaint” areas, and compare the occurrence of species and their relative concentrations.

Q11: Why is dust sample analysis more costly than air samples?

Dust samples are more prone to contain background materials that may be PCR inhibitors. For this reason, the analytical process is more labor intensive, requiring extensive sample purification and multiple dilutions, before analysis may begin.

Q12: Should I submit an outside sample?

Fungal population density outdoors has been observed to seasonally fluctuate yielding marked changes in species composition and densities. Due to the highly sensitive and specific attributes of this DNA-based technology, when outdoor samples are comparatively assessed with indoor data, particularly during seasonal increases of population density outdoors, the results may be skewed and significant indoor data may be masked when compared to outdoor data. Therefore, it is advisable to use “complaint” versus “non-complaint” areas for comparative purposes.

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Q13: Is Pure Earth licensed to perform this methodology?

YES! Pure Earth is licensed with the United States Environmental Protection Agency in a Cooperative Research and Development Agreement (CRADA). Furthermore, Pure Earth holds accreditation from the American Industrial Hygiene Association among other leading agencies, and adheres to ISO 17025, the most rigorous standards in the industry.