Pan-fungal PCR

Molecular detection and identification of fungi performed by fungal DNA extraction and molecular sequencing from patient specimens

General information

Turnaround time: 2-4 weeks. Performed on demand

Specimens: sterile sites, such as but not limited to blood, pleural fluid, CSF, deep tissue, biopsy, formalin-fixed paraffin-embedded (FFPE) materials from any site. Some respiratory specimens are accepted, e.g., BAL, bronchial washings, and other samples, e.g., from wound sites, burns. Sputum is not accepted unless a special case (please contact the laboratory prior to sending).

Sample type/container:

Blood: whole blood or EDTA tube, minimum volume 1 ml

Please DO NOT send blood in tubes other than the appropriate blood tubes described above; other tubes will be rejected.

Non-blood specimens, including fluid specimens, such as but not limited to pus, BAL, pleural fluid, peritoneal fluid are to be collected into appropriate UKCA/CE-marked sterile leakproof containers and transported at room temperature in sealed plastic bags.

Fixed paraffin sections (**preferred**): 2-3x thick (20 µm) consecutive sections placed together in a sterile container and transported at room temperature. Please also supply a Grocott-stained slide of 1x 4 µm section cut following the 2-3x thick sections as a quality control to assess fungal burden.

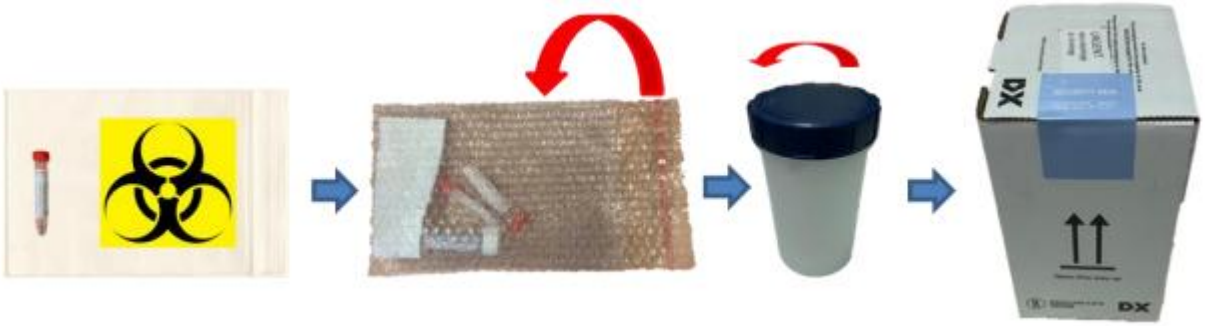
Fixed paraffin blocks: transported in a sterile container at room temperature. Please also supply Grocott-stained or D/PAS slides of the same material for reference.

For fixed materials, please also send the accompanying histopathology report.

Transportation

- Samples should be placed into a plastic Ziploc bag, sealed, and then placed into another sealed plastic Ziploc bag (preferably with a biohazard label on the outside), as shown below.

Category B transport boxes or an appropriate transport bag (i.e., one which adheres to regulations governing the transportation of diagnostic specimens) must be used for transport by road or between Manchester University NHS Foundation Trust sites (but are not necessary within Wythenshawe hospital grounds). Please see below for packaging requirements.



For more information - https://mrcm.org.uk/sample-collection/

Laboratory Information

Biological interval/clinical decision values:

The fungal ribosomal DNA Internal Transcribed Spacer (ITS) region, encompassing 18S, 5.8S and 28S rDNA, is analysed using 4 sets of sequencing primers.

Fungal identification is provided to species complex if possible.

Clinical Information

Indications for testing: clinically significant fungal infection, with negative culture and/or serology, or detection of fungal structures in sterile sites by histopathology in the absence of parallel fresh (un-fixed) sample.

Cases should be discussed with MRCM staff (Hive chat, mrcm@mft.nhs.uk, or mft.mrcm@nhs.net), who will advise.

Sanger sequencing is provided by Eurofins Genomics GmbH.

References:

Kurtzman CP, Fell JW, Boekhout T (eds). 2011. The Yeasts, a Taxonomic Study, Volume 1, Fifth Edition. Elsevier: London.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium et al. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 109(16):6241-6246; DOI: 10.1073/pnas.1117018109.

Schoch CL, Robbertse B, Robert V et al. 2014. Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi. Database 2014:bau061. doi:10.1093/database/bau061.

Vu D, Groenewald M, de Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol. 2019 Mar;92:135-154. doi: 10.1016/j.simyco.2018.05.001.

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(Last updated September 2026)