Great Ormond Street Hospital for Children
NHS Foundation Trust

Metagenomics Pathogen Detection Service

Metagenomics is a broad-range test that utilises deep sequencing to sequence total RNA and total DNA in a clinical specimen; this has the potential for un-targeted pan-pathogen detection.

Metagenomics is particularly useful in difficult-to-diagnose syndromes, such as encephalitis, in which an infectious aetiology is suspected but cannot be identified by routine methods.

In CSF, the sensitivity is similar to targeted real-time PCR for both RNA and DNA pathogens.

In tissue, the sensitivity for RNA viruses is similar to targeted real-time PCR. For DNA viruses, bacteria and fungi the sensitivity in tissue is reduced (approximately 100-fold) compared to targeted real-time PCR but comparable to pan-bacterial PCR (16S rRNA gene). Low level DNA pathogens in tissue may not be detected.

Please note that metagenomics has the potential to detect any infectious organism present in a patient's sample. On receipt of a metagenomics test request GOSH assumes patient consent for this diagnostic test has been sought; the responsibility for patient consent lies with the requesting clinician.

Please note this test is not yet accredited by UKAS against ISO 15189:2012.

Test Description	Specimen required	NHS Price	Non-NHS Price	Turnaround Time	Request Form
Metagenomics	Tissue biopsy (fresh/frozen or FFPE) CSF	£1269.85	£1635	2 weeks	Metagenomics request form

SPECIMEN REQUIREMENTS

Metagenomics is currently offered for tissue biopsies and CSF. The recommended specimen type is fresh tissue collected directly into RNALater.

See below for collection and storage requirements.

CSF
1 ml (minimum 500 µl) whole CSF (not filtered or centrifuged). We recommend CSFs are stored at −80 °C within 24 hours of collection (maximum 72 hours) to minimise RNA degradation. CSF is not stable at room temperature.

CSF collected with RNALater is not recommended due to poor sequence outcomes associated with dilution of the CSF.

Smaller volumes, sub-optimal storage or any pre-processing (e.g. filtering or centrifugation) will reduce ability to detect pathogens.

Fresh tissue - RNALater
Specimens must be collected directly into RNALater at the point of collection (e.g. in theatre); this is to rapidly stabilise RNA and prevent degradation. Specimens stabilised in RNALater can be stored at +4 °C for up to one month prior to shipping and are stable at room temperature for up to 1 week.

Ready to use pre-aliquoted RNALater can be purchased directly from Thermo Fisher Scientific (catalogue number AM7022). Aliquots of RNALater are also available on request from GOSH Microbiology/Virology labs.

Please note only RNALater has been validated for or processing methods therefore we do not advise the use of other RNA stabilisation solutions.

Fresh tissue - Frozen
Specimens that are not stabilised in RNALater should be immediately collected onto dry ice (e.g. in theatre) and stored at −80 °C prior to shipping. It is important to avoid any freeze-thaw cycles.

Fresh tissue - unstabilised
Tissue that has not been immediately stabilised in RNALater nor frozen is not recommended for metagenomics due to rapid degradation of RNA. Tissue in saline is not suitable.

Please be aware sub-optimal specimen collection, storage or transport will result in RNA degradation which will significantly reduce ability to detect RNA pathogens.

FFPE (formalin fixed paraffin embedded) tissue
8x 10 µm or 4 x 20 µm rolled sections

Please be aware the ability to detect low-level pathogens will be reduced in FFPE tissue compared to fresh tissue due to degradation of nucleic acid during formalin fixation. FFPE is a sub-optimal specimen type but can be processed in the absence of fresh tissue.

Specimen Transport

Tissue in RNALater:	Ambient temperature
FFPE:	Ambient temperature
Frozen tissue:	Dry ice
CSF:	Dry ice

Please send samples accompanied by request form to:
Virology Laboratory
Level 4, Camelia Botnar Laboratories
Great Ormond Street Hospital for Children
Great Ormond Street
London WC1N 3JH

Hays Dx: GOSH DX 6640203, Bloomsbury 91 WC

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Relevant publications arising from metagenomics service in our laboratory

1. Atkinson L, Lee JCD, Lennon A, Shah D, Storey N, Morfopoulou S, Harris KA, Breuer J and Brown JR (2023). Untargeted metagenomics protocol for the diagnosis of infection from CSF and tissue from sterile sites, *Heliyon*, **9**(9): e19854. (https://doi.org/10.1016/j.heliyon.2023.e19854)
2. Penner J, Hassell J, Brown JR, *et al*. (2023). Translating metagenomics into clinical practice for complex paediatric neurological presentations, *Journal of Infection*, in press.
3. Morfopoulou S. *et al.* (2023). Genomic investigations of unexplained acute hepatitis in children, *Nature*, **617**: 564–573. (https://doi.org/10.1038/s41586-023-06003-w)
4. de Vries JJC, Brown JR, Fischer N, Sidorov IA, Morfopoulou S, Huang J, Oude Munnink BB, Sayiner A, Bulgurcu A, Rodriguez C, Gricourt G, Keyaerts E, Beller L, Bachofen C, Kubacki J, Samuel C, Florian L,

Dennis S, Beer M, Hoeper DI, Huber M, Kufner V, Zaheri M, Lebrand A, Papa A, van Boheemen S, Kroes ACM, Breuer J, Lopez-Labrador FX, Claas ECJ (2021). Benchmark of thirteen bioinformatic pipelines for metagenomic virus diagnostics using datasets from clinical samples, *Journal of Clinical Virology*, **141**: 104908 (https://doi.org/10.1016/j.jcv.2021.104908)

5. Brown JR, Bharucha T and Breuer J (2018). Encephalitis diagnosis using metagenomics: application of next generation sequencing for undiagnosed cases. *Journal of Infection*, **76** (3): 225-240.
6. Bucciol G, Moens L, Payne K, Wollants E, Mekahli D, Levtchenko E, Vermeulen F, Tousseyn T, Gray P, Ma CS, Tangye SG, Van Ranst M, Brown JR, Breuer J and Meyts I (2018). Chronic Aichi virus infection in a patient with X-linked Agammaglobulinemia, *Journal of Clinical Immunology*, **38** (7): 748-752.
7. Morfopoulou S, Mee ET, Connaughton SM, Brown JR, Gilmour K, Chong WK, Duprex WP, Ferguson D, Hubank M, Hutchinson C, Kaliakatsos M, McQuaid S, Paine S, Plagnol V, Ruis C, Virasami A, Zhan H, Jacques TS, Schepelmann S, Qasim W, Breuer J (2016). Deep sequencing reveals persistence of cell-associated mumps vaccine virus in chronic encephalitis, *Acta Neuropathologica*, **133** (1): 139-147.
8. Lum SH, Turner A, Guiver M, Bonney D, Martland T, Davies E, Newbould M, Brown J, Morfopoulou S, Breuer J, Wynn R (2016). An emerging opportunistic infection: fatal astrovirus (VA1/HMO-C) encephalitis in a pediatric stem cell transplant recipient, *Transplant Infectious Disease*, **18**(6): 960-964
9. Morfopoulou S, Brown JR, Davies EG, Anderson G, Virasami A, Qasim W, Chong WK, Hubank M, Plagnol V, Desforges M, Jacques TS, Talbot PJ, Breuer J (2016). Coronavirus HCoV-OC43 Associated with Fatal Encephalitis, *New England Journal of Medicine*, **375**(5): 497-8.
10. Duncan JAD, Mohamad SMB, Young DF, Skelton AJ, Leahy TR, Munday DC, Butler KM, Morfopoulou S, Brown JR, Hubank M, Connell J, Gavin PJ, McMahon C, Dempsey E, Lynch NE, Jacques TS, Valappil M, Cant AJ, Beuer J, Engelhardt KR, Randall RE and Hambleton S (2015). Human IFNAR2 deficiency: Lessons for antiviral immunity, *Science Translational Methods*, **7** (307).
11. Brown JR, Morfopoulou S, Hubb J, Emmett WA, Ip W, Shah D, Brooks T, Paine SML, Anderson G, Virasami A, Tong CY W, Clark DA, Plagnol V, Jacques TS, Qasim W, Hubank M, Breuer J (2015). Astrovirus VA1/HMO-C: an increasingly recognised neurotropic pathogen in immunocompromised patients, *Clinical Infectious Diseases*, **60** (6): 881-888.