**What is Advanced Molecular Pathology Laboratory**

The Advanced Molecular Pathology Laboratory (AMPL) is a joint effort between the Institute of Molecular and Cell Biology (IMCB) and Singapore Health Services (SingHealth). This world class facility provides a wide range of pathology-related services in basic and translational research, therapeutic target validation and drug safety evaluation in biomedical research community and industry.

The aims of this facility are as follows:

- To provide pathology services as a one-stop facility for pharmaceutical and biotechnology companies who engage in exploratory, preclinical and early clinical studies
- To provide veterinary clinical diagnosis to the pet health sectors
- To provide *invitro* toxicology services (alternate to animal testing) to pharmaceuticals, cosmetics and agrochemical companies
- To generate high quality tissue bio resources for the research community and biomedical industry, in collaboration with the SingHealth Tissue Repository (STR)

**Core Strengths of the AMPL**

The core strength of AMPL in animal research services includes GLP-grade animal necropsy, histology services, immunohistochemistry, in situ hybridization, image analysis, veterinary pathology evaluation and *invitro* toxicology testing. At AMPL@SingHealth, researchers will have access to a CAP-compliant diagnostic facility with a variety of techniques necessary for human tissue-based research including histotechnology, histopathology consultation, immunodiagnostics and molecular assays. The AMPL is also closely linked to the SingHealth Tissue Repository (the largest human tissue research biobank in Singapore), researchers in non-human primate research and research institutions with cutting-edge translational research capabilities.

**Why Should Researchers and Industry Use Our Services**

To ensure the highest professional standards, AMPL has a team of pathologists and scientists, supported by experienced medical and laboratory technologists. Our veterinary pathologists are either board certified by American College of Veterinary Pathologists (ACVP) or American Board of Toxicology (ABT) with decades of experience in the pharmaceutical industry. AMPL is headed by an experienced histopathologist with local license and international qualifications. As a research service provider, our charges are competitive and we constantly ensure our services are cost-efficient. The AMPL is a unique research service provider that brings together expertise across multiple disciplines to support researchers and industrial clients. We also provide consultation for project design, applications to IRB and tissue access committees. Clients can expect 100% quality control from AMPL, which also functions as a conduit for knowledge, experience and clinical resources from around Singapore.
**Rodent Necropsy and Veterinary Pathology**

Researchers will have access to the expertise of our board-certified veterinary pathologists and a team of well-trained laboratory technicians who can perform rodent necropsies at our GLP-accredited histopathology laboratory or in client facilities. At AMPL, photography of gross lesions is performed with the Milestone MacroPATH D Macro Digital Imaging System and the Olympus Stereo microscope.

We provide dissection, macroscopic examination and processing of a wide range of tissues originating from human, Zebrafish to animal (both rodents and non-rodents) sources for molecular research as well as regulatory toxicology studies. We offer various embedding techniques such as paraffin, MMA and OCT.

Over the years, AMPL has established a reputation for excellence in immuno-histochemical techniques that include antibody evaluation/optimization on cytological specimens; paraffin embedded archival material and tissue microarrays.

Upon request, we can also perform nucleic acid extraction from laser micro-dissected cells and tissue sections.
Pathology and Histological Consultation

In terms of clinical histopathology, researchers will have access to the expertise of a UK-qualified histopathologist and scientist, supported by a team of Pathology Associates, who can provide consultation for interpretation of histology, grading of immunostains and classification of human neoplasms according to standard or protocol-defined criteria. We can also assist with selection of material for downstream applications, such as comparative genomic hybridisation (CGH) and SELDI-mass spectrometry.
**Immunodiagnostics**

The facility is able to provide a full range of immunodiagnostic techniques ranging from single-label immunostaining to multi-color immunolabelling to demonstrate co-expression of two or more molecules in a particular cellular subset.

TUNEL assays for apoptosis and in situ hybridization (ISH) assays for detection of viral RNA can also be performed, e.g. EBER for EBV infection, as well as detection of kappa and lambda mRNA for the determination of light chain restriction. In addition, the AMPL will also evaluate new antibodies for research and diagnostic purposes.
Tissue Microarray Construction

Tissue microarray (TMA) is a technique that places numerous tiny cores of tissue samples on a single microscope slide and is a useful tool for high-throughput analysis of protein expression across a large number of samples. TMA technology is also suitable for analysis of RNA and genetic alterations by the ISH, CISH and FISH techniques.

The AMPL constructs and stocks tissue microarrays derived from a variety of tissue types and neoplasms. All tissue arrays are produced under the supervision of a pathologist, thereby ensuring their quality. In addition, dedicated tissue arrays can be custom-made for a particular study upon request by researchers. AMPL also constructs cellular arrays derived from malignant effusions and cell lines.
Cellular Array Construction

The AMPL is able to make isolated cell preparations from paraffin embedded archival material, fresh tissues as well as cytological specimens for immunocytochemistry, FISH and immuno-FISH experiments. By constructing cell blocks, the facility also produces and stocks cellular arrays from a variety of human neoplasms.

**Extraction of cells for immunoFISH**

- Extract tissue core from selected area
- De-wax core in xylene and rehydrate with decreasing concentrations of ethanol
- Homogenize tissue manually with spatula
- Add PBS and centrifuge for 6000rpm for 10 mins
- Discard supernatant. Re-suspend in PBS.
- Filter through 50nm nylon mesh
- Centrifuge for 6000 rpm for 10 mins
- Discard supernatant. Re-suspend in PBS.
- Pipette onto glass slide. Air dry.
- Immunofluorescence labelling with AlexaFluor350
- FISH with overnight incubation

**Images**

- a) Ki67/BCL2 break-apart
  - Mantle cell lymphoma
  - Burkitt lymphoma
  - DLBCL
  - Tonsil
  - Follicular lymphoma
  - DLBCL

- b) 50-spot cellular array
**Fluorescence In Situ Hybridisation (FISH)**

AMPL has the capacity to perform FISH and whole chromosome painting with homemade and commercial reagents. In recent years, interphase FISH has become a valuable tool in the investigation of genetic alterations in cytological and histological specimens, both in diagnostic and research settings.

In addition, AMPL is also able to perform immuno-FISH (FICTION technique), which combines the power of FISH to detect genetic alterations with lineage identification of cells of interest by immunophenotype.
Laser Capture Micro-dissection

Laser capture micro-dissection is a tool for extracting cellular subsets from tissue material for downstream applications in molecular biology (e.g. gene expression profiling, comparative genomic hybridisation, MALDI/SELDI-TOF). Researchers can have access to qualified staff trained in the use of laser micro-dissection with the Arcturus LCM, Leica LMD and Carl Zeiss’ PALM Microbeam systems.
**Nucleic Acid Extraction**

As a service to researchers, AMPL will also extract DNA and RNA from frozen and FFPE material and perform quality control analyses on the extracts. Apart from spectrophotometry and gel electrophoresis, calculation of the RIN index can be performed using the Agilent Bioanalyzer.
Antibody Evaluation and Probe-Making Services

The AMPL provides services for researchers in the healthcare clusters and commercial companies to screen antibodies and evaluate their staining characteristics in normal and neoplastic tissues of human and animal origins. In addition, we will be able to design, construct and validate for researchers, locus-specific FISH probes against genes of interests or region-specific probes to verify regions of amplifications/deletions as identified by CGH.
At AMPL, Leica SCN400 scanner and Metasystem scanner provide a perfect pathology workstation for the assessment of biomarker expression by fluorescent and bright-field microscopy. They add the ability to store, manage, analyze, and report on digital images in preclinical and clinical research.

Scanned images are viewed through Slidepath Digital Imaging Hub (Leica Microsystems). This service allows secure data sharing, tele-pathology and conferencing through a secure web browser from anywhere in the world. It also facilitates the archiving and sharing of slides and capturing of images for publication. Predefined image analysis protocols measure the various parameters such as whole Cell Quantification that includes nuclear, membrane, and cytoplasmic analysis.

Ki-67 positive nuclei to quantify cell proliferation in small intestine: Rat small intestine stained with hematoxylin and anti-Ki-67 antibody (DAB) is analyzed with nuclear analysis tool. Red nuclei are positive for DAB; blue nuclei are negative in digitalized image.
Micro vessel density area measurement in rat heart

Rat heart tissue is stained with hematoxylin and CD31 for measuring the micro vessel density. It calculates the vessel area, lumen size and perimeter of vessel. (*"Courtesy- Dr. Udi Sarig: The regenerative medicine initiative in cardiac restoration therapy under the Singapore Technion Alliance in Research and Technology (START)"")

For very specialized tasks that require cutting-edge image analytic solutions, we provide personalized service with a team of experts from the Bioinformatics Institute who developed artificial intelligent software to mimic the human visual system. Our intelligent visual system is particularly suited for high-level pattern recognition work. Cellular pattern recognition is of emerging importance in clinical and biological research.
Partnership with Institute of Medical Biology – Microscopy Unit (IMU)

IMU is a core technology platform of the Institute of Medical Biology whose mission is to provide light and electron microscopy equipment along with image processing and analysis software and the expertise to facilitate research in molecular, cell and developmental biology. A full description of IMU can be found on the IMU website (http://www.imb.a-star.edu.sg/imu/index.php).

As well as standard widefield and confocal microscopy IMU focuses on seven areas of advanced imaging:

1. 3D imaging including light sheet imaging
2. F-techniques (FRET, FRAP, FLIM and FCS)
3. High content imaging
4. Live-cell imaging
5. Super-resolution microscopy (PALM, dSTORM and SIM)
7. Image analysis & processing

IMU’s particular focus is to use optical imaging to investigate cell and developmental biology. We are also keen to investigate human 3D cell culture systems (e.g. human skin).

A scanning electron microscopy (SEM) image of a neuroblastoma cell. Sample preparation and image acquisition by Bu Wenyu (Sohail Ahmed’s lab) and Low Kay En (Jeol).
Training Researchers, Clinicians and Pathologists at AMPL @SingHealth

Training is an important aspect of the AMPL. The facility conducts lectures and workshops for researchers, medical students, clinicians and pathologists who wish to acquire the technical expertise for tissue-based research.

Training attachments and workshops are regularly conducted in various areas:

- Basic histo-technology: microtomy, hematoxylin/eosin staining, making cryostat sections
- Basic immunohistochemistry: immunostaining of frozen sections, paraffin embedded tissues and cytological specimens
- Multi-label immunohistochemistry: double and triple immunofluorescence labeling, combining immune-peroxidase with immunofluorescence staining
- Fluorescence in situ hybridization (FISH): performing FISH on metaphase spreads, blood smears and cytological specimens, frozen sections and paraffin embedded tissue sections
- Tissue microarray technology: constructing tissue microarrays from archival paraffin embedded material
- Laser microdissection

Services and Charges

There are two components to the charge: (1) reagent charge and (2) service charge. The service charge will be determined after discussion with the researcher and will depend on the nature of the protocol, the possibility of batching the tests, complexity of the procedure and the number of specimens. Please contact us for details.
A/Prof Tan Soo Yong obtained his medical qualifications from the National University of Singapore and thereafter underwent postgraduate training in Forensic Pathology under the late Prof Chao Tzee Cheng in Singapore and Prof Michael Green in the University of Sheffield, United Kingdom. Qualifying in both forensic and histopathology, he is a Fellow of the Royal College of Pathologists (UK) and a Diplomate of the Society of Apothecaries of London. Dr Tan obtained his PhD from Oxford University, working in the field of haematopathology. He is currently Associate Professor in Duke-NUS Graduate Medical School, a Senior Consultant and Clinician Investigator in the Department of Pathology, Singapore General Hospital and Senior Consultant to the Ministry of Health, Singapore. Apart from heading the Advanced Molecular Pathology Laboratory (AMPL), he also holds concurrent appointments as Director of the Singhealth Tissue Repository, Senior Principal Investigator at the Institute of Cell and Molecular Biology (IMCB), Chief Examiner in Pathology, Chairman of the Residency Advisory Committee for Pathology and Visiting Professor to University Malaya. He also sits on the Editorial Board of two pathology journals (Journal of Clinical Pathology, Biobanking and Biopreservation). Dr Tan has multiple administrative and advisory roles including Consultant to Johnson & Johnson, Inc., member of the Diagnostic Hotspot Advisory Panel, Exploit Technologies, Singapore’s representative to the Advisory Board of the IAEA, Member of Advisory Board, Roche Ventana, Chairman of the Biobanking Subcommittee, Asian Network of Research Resource Centres (ANRRC) and International Advisor (West Pacific) of the Royal College of Pathologists (UK). His current research interest is in the pathology of NK/T cell lymphoma [Cancer Discov. 2012 Jul;2(7):591-597] and Type II Enteropathy-associated T-cell lymphoma. [Leukemia. 2011 Mar;25(3):555-7; Leukemia. 2013 Feb 12. doi:10.1038/leu.2013.41.]
Dr. Ravisankar Rajarethinam received his basic degree in Veterinary Medicine (BVSc) and specialized in Veterinary Pathology (MVSc) from Tamil Nadu Veterinary and Animal Sciences University, India. He obtained his PhD in Veterinary Pathology from the Indian Veterinary Research Institute, India. He is also a board-certified toxicologist (DABT) by American Board of Toxicology. He started his professional career as a Toxicopathologist at Glenmark Pharmaceuticals, Mumbai, India and collaboratively worked with multidisciplinary departments to support the investigation of efficacy and toxicity of new drugs. He worked as a Senior Scientist at Ranbaxy Research Laboratories, New Delhi, India and was involved in preclinical research of new chemical entities in different animal models of inflammation, metabolic disorders, immune mediated diseases and oncology. Later, he served as a Lead Investigator, heading the Pathology Department at a GLP-certified CRO Company, Syngene International Ltd, Bangalore, India and focused mainly on preclinical animal studies for the safety evaluation of drugs, chemicals and biologicals. At present, Dr. Ravi oversees the laboratory activities at AMPL that include technical support for basic and translational research as well as safety assessment of drugs.

Dr. Chee Bing Ong was awarded the National Science Scholarship by Agency for Science, Technology and Research (A*STAR) in 2002 and obtained his Bachelor of Veterinary Science (with first-class honours) from The University of Melbourne, Australia. He worked as a laboratory animal veterinarian in Biological Resource Centre (A*STAR) after graduation. Thereafter, he pursued a residency in Veterinary Anatomic Pathology in the Diagnostic Center for Population and Animal Health, Michigan State University, United States. During his residency, he gained extensive experience in veterinary pathology, necropsies, surgical histopathology of various animal species and also completed a Master of Science in Pathobiology involving the study of respiratory toxicology in laboratory mice. Dr Ong is board certified in Anatomic Pathology by the American College of Veterinary Pathologists (ACVP), and is currently a Veterinary Pathologist in The Institute of Molecular and Cell Biology (IMCB), A*STAR, Singapore.
Dr Susan Hue Swee Shan was awarded with MBBS-PhD Scholarship by Agency for Science, Technology and Research (A*STAR) in 2000. She obtained her medical qualifications from National University of Singapore and her PhD from Imperial College London, under the Department of Immunology. She undertook further postgraduate specialty training and qualified as a histopathologist in 2015. She is a Fellow of The Royal College of Pathologists of Australasia (FRCPA).

She currently holds dual appointments, serving her clinical role as Associate Consultant in the Department of Pathology, National University Hospital (NUH) and as a Research Clinician in Advanced Molecular Pathology Laboratory (AMPL)/Institute of Molecular and Cell Biology (IMCB). Her research interests are lymphoma pathology and tumour immunology. At AMPL, IMCB, she oversees the pathology evaluation of human samples.

Miss Poh Suat Fang received her diploma in Biomedical Science (2008) and graduate degree in Business (2012). She has been working at Institute of Molecular and Cell Biology, Advance Molecular Pathology Laboratory since 2008. She worked as a senior laboratory officer for the first 4 years in the laboratory before taking up the role in the Quality Assurance (QA) Unit. She is involved in the routine work from rodent necropsy to tissue processing, sectioning and staining to quality control. As an Assistant QA Manager, she is responsible in assuring the GLP compliance of laboratory activities in both the histopathology laboratory and In-Vitro Toxicity Facility, reviewing document for GLP compliance and involved in verification of documents. She also conducts audits that include process-based, study-based and facility-based audits and hosts the client and regulatory inspection and prepares responses to the inspection reports.
## How to Contact Us

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<thead>
<tr>
<th>Animal Tissue-Based Bioresources and Research Services</th>
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<tbody>
<tr>
<td>AMPL@Biopolis</td>
<td>AMPL@SingHealth</td>
</tr>
<tr>
<td>61 Biopolis Drive, Proteos Building Level 6</td>
<td>The Academia, Discovery Tower</td>
</tr>
<tr>
<td>Singapore -138673</td>
<td>Level 10, 20 College Road,</td>
</tr>
<tr>
<td>Telephone: (65) 6586 9858</td>
<td>Singapore -169856</td>
</tr>
<tr>
<td>Email: <a href="mailto:AMPL@imcb.a-star.edu.sg">AMPL@imcb.a-star.edu.sg</a></td>
<td>Telephone: (65) 6576-7116</td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:ampl@singhealth.com.sg">ampl@singhealth.com.sg</a></td>
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