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ENVIRONMENTAL IMPACT OF PATHOLOGY LABORATORIES. SENTINEL LYMPH NODE DIAGNOSTIC METHODOLOGIES: A CASE STUDY

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Abbreviations

ALND: axillary lymph node dissection

AUC: area under the curve

BSI: British Standards Institution

CBA: Cost-Benefit Analysis

CFCs: chlorofluorocarbons

CK19: cytokeratin 19

CML: Centrum voor Milieukunde Leiden

CML-IA: CML Impact Assessment

CO_2eq: CO_2 equivalents

DU: Declared Unit

EF: Environmental Footprint

EIA: Environmental impact evaluation

ELCD: European Reference Life Cycle Database

EPA: US Environmental Protection Agency

ERA: Environmental Risk Assessment

ESP: Environmental Priority Strategies

FU: functional unit

GIS: Geographic Information Systems

GWP100a: Global warming potential 100 years

H&E: hematoxylin and eosin

IDEA: Inventory Database for Environmental Analysis

IHC: immunohistochemistry

ILCD: International Reference Life Cycle Data System

IPCC: Intergovernmental Panel on Climate Change

IRCCS: Istituto di Ricovero e Cura a Carattere Scientifico

ISO: International Organization for Standardization

JEMAI: Environmental Management Association for Industry

JRC: European Commission's Joint Research Center

LAMP: loop-mediated isothermal amplification

LCA: Life Cycle Assessment

LCI: life cycle inventory

LCIA: life cycle impact assessment

MCA: Multi-Criteria Analysis

OEF: Organizational Environmental Footprint

ODP: ozone layer depletion

PEF: Product Environmental Footprint

PP: Polypropylene

PPV: positive predictive value

RNA: ribonucleic acid

SETAC: Society of Environmental Toxicology and Chemistry

SLNB: Sentinel lymph node biopsy

SNL: sentinel lymph node

TAB: tissue allocation bias

TRACI: Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts

UNEP: United Nations Environment Programme

UV: ultraviolet

VOC: volatile organic compounds

WBCSD: World Business Council for Sustainable Development

WHO: World Health Organization

WRI: World Resources Institute

WWF: World Wildlife Fund

Section 1 : Introduction

Throughout history, humans have been active agents in shaping the natural world to meet their needs. From early agricultural practices to modern industrialization, human activities have had a profound impact on the environment. This ability to modify our surroundings has been a defining characteristic of our species, but it has also led to significant environmental challenges[1].

One of the most significant consequences of human activity has been the creation of an environment that is better suited for our survival[2]. Advances in agriculture, medicine, and technology have allowed us to produce more food, live longer, and protect ourselves from diseases. These improvements have led to a sharp increase in the global population, which has further intensified our impact on the environment[3].

The combination of human ingenuity and population growth has made us the only species capable of large-scale environmental alterations[4]. The burning of fossil fuels is the most significant contributor, accounting for approximately 75% of total greenhouse gas emissions. Agricultural activities, including livestock methane emissions, cultivation, and deforestation, contribute around 10-12%. Deforestation alone accounts for approximately 10% of total emissions. Industrial processes, such as manufacturing and construction, release greenhouse gases like carbon dioxide and nitrous oxide, contributing roughly 5%. Waste disposal and transportation also contribute to emissions, with approximately 3% and 5%, respectively[5].

Furthermore, improvements in living conditions and advancements in healthcare have contributed to increased human longevity. As a result, the proportion of the elderly population has grown, leading to a greater demand for long-term care services[6]. This creates a cyclical dynamic in which enhanced healthcare extends life expectancy, thereby generating a continued and increasing need for healthcare support in older age[7].

Modern healthcare systems are widely recognized for their heavy reliance on single-use items[8], often made from plastics and other non-biodegradable materials. This dependence contributes to growing environmental challenges[9], particularly in terms of disposal and waste management. Consequently, there is an increasing need for deliberate and sustained efforts to reduce this reliance and promote more sustainable practices within the healthcare sector[10] [11].

The energy-intensive nature of healthcare[12] is another point of concern, encompassing everything from hospital operations to the production and sterilization of medical equipment, it further exacerbates the healthcare environmental footprint[13].

Considering the ever-growing human population and the consequent need for health care it is important to consider the impact of those large scale activities on the environment in which we live.

1 Chapter : Laboratories Environmental Impact

1.1 Environmental Challenges

Human activities have significantly impacted the environment, leading to a range of detrimental consequences. While the extent of these impacts varies across regions and time periods, it is evident that our actions have had a profound effect on the planet.

One of the most significant human-induced environmental problems is **climate change**. This global issue is primarily driven by the release of greenhouse gases, a group of heat-trapping gases that include carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and fluorinated gases. These gases are released in large quantities through the burning of fossil fuels, deforestation, and various industrial processes. Greenhouse gases accumulate in the atmosphere trapping heat and leading to increased temperatures. [14]This enhanced greenhouse effect has continued to intensify in recent years[15] and has led to rising global temperatures, accelerated melting of ice sheets, sea-level rise, and a noticeable increase in the frequency and intensity of extreme weather events.

According to the **Intergovernmental Panel on Climate Change (IPCC)**, global temperatures have increased by approximately 1.1°C since the late 19th century, and the rate of warming is accelerating [16].

Another major environmental issue is **biodiversity loss**. Human activities, such as habitat destruction, pollution, and over-exploitation of resources, have led to the decline and extinction of countless species[17] [18]. Deforestation, in particular, has had a devastating impact on biodiversity, as it destroys habitats and disrupts ecosystems. The **World Wildlife Fund (WWF)** estimates that global wildlife populations have declined by an average of 73% since 1970[19] .

Pollution is also a pressing environmental concern. Air pollution, caused by the burning of fossil fuels, industrial emissions, and vehicle exhaust, can have serious health consequences, including respiratory diseases, heart disease, and cancer[20]. Water pollution, resulting from industrial

discharges, agricultural runoff, and municipal wastewater, can contaminate drinking water sources and harm aquatic ecosystems[21]. Soil pollution, caused by industrial waste, agricultural chemicals, and improper waste disposal, can degrade soil quality and reduce agricultural productivity[22] [23].

Resource depletion is a significant environmental problem. The over-consumption of resources, such as fossil fuels, minerals, and timber, has led to their depletion and degradation. This can have serious consequences for both human well-being and the environment. For example, the depletion of fossil fuels contributes to climate change, while the over-exploitation of forests can lead to deforestation and biodiversity loss[24] [25].

In addition to these global challenges, human activities have also led to a range of **local environmental problems**. These include habitat fragmentation, invasive species, and desertification. Habitat fragmentation, caused by urbanization, agriculture, and infrastructure development, can isolate populations of species and reduce biodiversity[26]. Invasive species, introduced to new environments by human activities, can out-compete native species and disrupt ecosystems[27]. Desertification, caused by overgrazing, deforestation, and climate change, can lead to land degradation and loss of productivity[28].

In conclusion, human activities have had a profound impact on the environment, leading to a range of serious problems. Addressing these challenges will require a concerted effort to reduce our environmental footprint, promote sustainable development, and protect biodiversity.

1.1.1 Health care

Healthcare activities, while essential for human well-being, can also have a significant environmental impact. Hospitals and clinics consume substantial amounts of resources, including energy, water, and materials, and generate significant amounts of waste. These activities can contribute to climate change, pollution, and resource depletion.

Healthcare facilities are often **energy-intensive**, requiring large amounts of electricity to power equipment, heat and cool buildings, and sterilize medical instruments. This energy consumption can contribute to greenhouse gas emissions and climate change. According to the **World Health Organization (WHO)**, healthcare facilities account for approximately 4.5% of global greenhouse gas emissions[29] [30].

Healthcare facilities generate a **significant amount of waste**, including medical waste, pharmaceuticals, and packaging materials. Improper disposal of this waste can pose risks to human health and the environment[31] [32]. Medical waste, such as syringes, needles, and contaminated dressings, can contain infectious pathogens and requires careful handling and disposal[33] [34].

Pharmaceuticals can contaminate water sources and soil, and packaging materials, such as plastic and cardboard, contribute to waste streams.

Healthcare activities, such as patient care, diagnostic procedures, and cleaning, can consume **large quantities of water**[35] [36]. A study 2020 revealed an estimated range of between 103 m3/bed/year and 458 m3/bed/year for Italian hospitals[37]. This can put a strain on water resources, especially in areas with limited water availability.

In addition to energy consumption, waste production, and water use, healthcare activities have a broader environmental footprint. This includes the emission of greenhouse gases and air pollutants, the contamination of water bodies, the use of scarce water resources and the degradation of ecosystems through land use changes. Furthermore, emissions from the transportation of patients, staff, and medical supplies, as well as from global supply chains that produce pharmaceuticals and equipment, contribute significantly to the sector's overall environmental impact[38].s

1.1.2 Clinical laboratories

Clinical laboratories, often overlooked in discussions of environmental sustainability, play a vital role in modern healthcare. However, their operations can have a significant environmental footprint due to factors such as instrument energy consumption, maintenance requirements, waste generation, and the use of potentially toxic reagents. [39] [40] [41].

Energy Consumption: Clinical laboratory equipment, such as analyzers, centrifuges, and refrigerators, are energy-intensive. The continuous operation of these instruments, coupled with the need for temperature-controlled environments, contributes substantially to energy consumption. Moreover, the increasing use of advanced diagnostic techniques, such as molecular biology and genomics, further exacerbates energy demands.

Waste Generation: Clinical laboratories generate a significant amount of waste, including bio-hazardous materials, chemicals, and plastics. The proper disposal of this waste is crucial to prevent

environmental contamination and protect public health. However, the management of laboratory waste can be challenging and costly.

Reagents Use: Many reagents used in clinical laboratories are potentially toxic or hazardous to the environment. The disposal of these reagents requires careful handling and specialized treatment to prevent contamination of water bodies and soil.

1.2 Clinical Pathology Laboratories

Clinical pathology laboratories provide diagnostic information that aids in the diagnosis, treatment, and prevention of diseases. These laboratories analyze a wide range of biological samples, including blood, urine, tissue, and other bodily fluids, to identify abnormalities and assess the overall health of an individual.

The work of clinical pathology laboratories is essential for diagnosis of diseases, monitoring disease progression, screening for diseases, preventing the spread of infectious diseases, and contributing to medical research[42] [43]. A multitude of different functions performed by clinical laboratories are needed for the diagnostics procedures routinely performed in an hospital, some of the most common are:

Hematology is the study of blood and its components. Hematology procedures include complete blood count, differential count, coagulation tests, and blood smears.

Complete blood count is a comprehensive test that measures red blood cells, white blood cells, platelets, hemoglobin, hematocrit, and other blood parameters. Differential count is a test that counts the different types of white blood cells to help diagnose blood disorders. Coagulation tests measure blood clotting factors to diagnose bleeding disorders and monitor anticoagulant therapy. Blood smears are microscopic examination of blood cells to identify abnormalities such as anemia, leukemia, and infections.

Microbiology is the study of microorganisms. Microbiology procedures include culture and sensitivity testing, Gram stain, acid-fast stain, and molecular diagnostics.

Culture and sensitivity testing is a procedure used to grow microorganisms from samples and test their susceptibility to antibiotics. Gram stain is a staining technique used to differentiate bacteria into two major groups: Gram-positive and Gram-negative. Acid-fast stain is a staining technique used to identify acid-fast bacteria, such as Mycobacterium tuberculosis. Molecular diagnostics use DNA or RNA analysis to identify microorganisms.

Histopathology is the study of tissues. Histopathology procedures include tissue biopsy, tissue processing, staining, and microscopic examination.

Tissue biopsy is a procedure used to obtain tissue samples for microscopic examination. Tissue processing is a series of steps used to prepare tissue samples for staining and examination. Staining is a procedure used to color tissue samples to make cellular structures visible. Microscopic examination is a procedure used to examine tissue samples under a microscope to identify abnormalities.

Molecular Pathology characterize tissue in detail. Molecular pathology procedure include DNA or RNA assay to obtain detailed characterization of pathological specimens.

DNA analysis detect mutation in genes of interests that are known for their pathological implications . RNA analysis focuses on the expression levels of genes.

Cytology is the study of cells. Cytology procedures include Pap smear, fine needle aspiration, and fluid analysis.

A Pap smear is a test used to screen for cervical cancer. Fine needle aspiration is a procedure used to obtain cells from a mass or lesion for examination. Fluid analysis is a procedure used to examine fluids such as pleural fluid, peritoneal fluid, and cerebrospinal fluid for abnormalities.

Toxicology is the study of poisons. Toxicology procedures include drug screening and poisoning testing. Drug screening is a procedure used to detect the presence of drugs of abuse in the body. Poisoning testing is a procedure used to detect the presence of toxins and poisons in the body.

1.3 Surgical Pathology Laboratories

In this study, we aim to investigate the environmental impact of a widely utilized type of pathology laboratory: the surgical pathology laboratory, where histological analyses are performed. These laboratories specialize in the **examination and diagnosis of tissue samples obtained from patients during surgeries or other medical procedures**. They carry out various histological evaluations, which are critical for diagnosing a range of medical conditions, including cancer and other diseases.

While previous studies on the environmental impact of clinical laboratories have primarily focused on simpler, automated tests,such as blood cell counts and biochemical analyses of serum and urine[44] [45] or the reduction of toxic chemicals usage [46] providing highly valuable insights and laid a strong foundation for sustainable practices.

Moreover, in recent years, there have been new studies specifically on surgical laboratories, highlighting their distinct environmental challenges and contributions to healthcare-related emissions. For instance, Gordon et al. (2021) [47]conducted a comprehensive life cycle assessment of gastrointestinal biopsy processing. Their findings revealed that while per-case emissions were relatively modest (0.29–0.79 kg CO_2eq), the cumulative impact of millions of such procedures performed annually in the U.S. is substantial.

Similarly, Béchu et al. (2024) [48]assessed the total carbon footprint of a French surgical pathology laboratory, identifying laboratory input, particularly reagents and plastic consumables, as the main sources of emissions.

In a more solution-oriented approach, Rullier et al. (2025)[49] implemented and validated a formalin recycling system in routine pathology practice. This initiative not only reduced formalin consumption by 26% but also maintained diagnostic quality while achieving measurable reductions in CO_2 emissions and chemical toxicity.

Together, these recent investigations represent a crucial shift toward recognizing the specific environmental impact of surgical pathology labs. They demonstrate the value of combining

footprint assessments with practical interventions and set a precedent for incorporating sustainability into laboratory operations and diagnostic protocols.

However, although the environmental impact of hospital waste disposal and other areas of healthcare management has been extensively studied in diverse settings, the specific environmental footprint of surgical pathology laboratories remains a new and under-explored area[50].

To further contribute to this emerging field, we evaluated the environmental impact of one of the most routinely performed procedures in histopathology laboratories: the diagnostic analysis of a sentinel lymph node. Additionally, we compared the conventional histological methodology with a novel molecular alternative, aiming to broaden the perspective on the subject.

2 Chapter : Sentinel lymph Node Diagnostics

2.1 Lymphatic System

2.1.1 Metabolic function

The lymphatic system is an intricate and highly specialized network of vessels, nodes, and associated organs that plays a vital role in maintaining immune surveillance, fluid homeostasis, and the removal of interstitial waste.

Lymphatic vessels are delicate, endothelial-lined conduits that originate in the interstitial spaces of tissues that gradually converge to reintroduce the drained fluids into the bloodstream. Lymph is composed of immune cells as well as cellular debris, pathogens, and other foreign materials filtered from the tissues.

Lymph nodes are strategically distributed in clusters throughout the body—particularly in the cervical, axillary, inguinal, and mediastinal regions—and function as immunological checkpoints, filtering lymph as it drains from specific anatomical areas. These regions correspond to distinct drainage basins, each representing a defined territory of interstitial fluid collection. Within each basin, lymph is directed through a dedicated network of regional lymph nodes.

These encapsulated, bean-shaped structures house populations of B cells, T cells, and antigen-presenting cells, and function as filtration units, capturing and processing pathogens, apoptotic cells, and other particulate matter. Through coordinated immune responses within these nodes, the body initiates adaptive immunity and prevents the systemic spread of infection[51].

2.1.2 Role in cancer diagnostics

When cancer cells spread from a primary tumor, they often travel through the lymphatic system to other parts of the body.

The **sentinel lymph node(SLN)** is the first lymph node in the drainage basin that receives cancer cells from a primary tumor. It acts as a sentinel, or guard, at the entrance to a region of the lymphatic system. By examining the sentinel lymph node thought **serial sectioning**, **hematoxylin and eosin (H&E) staining**, and **immunohistochemistry (IHC)**,, doctors assesses the presence and extent of metastatic involvement within the node.

If cancer cells are found in the sentinel lymph node it supports a diagnosis of nodal involvement, which has significant implications for tumor staging, prognosis, and treatment planning, including decisions regarding adjuvant therapy. However, if the sentinel lymph node is negative for cancer cells, it does not necessarily mean that the cancer has not spread. Additional tests may be needed to confirm the stage of the cancer[52].

2.1.3 Diagnostic analysis

Sentinel lymph node biopsy (SLNB) is a minimally invasive surgical procedure used to identify and remove the sentinel lymph node, the first lymph node in a drainage basin that receives cancer cells from a primary tumor. This procedure offers several advantages over traditional **axillary lymph node dissection (ALND)**, which involves removing a larger number of lymph nodes. These procedures are performed in a multitude of cancer types in which there is a risk of spread in the lymphatic system, in this instance we will focus on SLNB in breast cancer diagnosis [53].

SLNB is a less invasive procedure than ALND, leading to less pain, a shorter recovery time, and fewer complications. By reducing the number of lymph nodes removed, SLNB can help to preserve lymphatic function, which is important for maintaining fluid balance and preventing lymphedema. SLNB can accurately determine whether cancer cells have spread to the lymph nodes, which is essential for staging the cancer and determining the appropriate treatment plan. SLNB can be more cost-effective than ALND, as it requires less surgical time and fewer resources[54].

While SLNB has become a standard of care for many types of cancer, such as breast cancer and melanoma, it may not be appropriate for all patients. The decision to perform SLNB should be made on an individual basis, taking into account the patient's specific circumstances and the type of cancer being treated[55].

The SLNB procedure generally comprises the following steps:

1. The patient undergoes anesthesia administration—either general or local, depending on the clinical context—to ensure procedural comfort and immobility.
2. A radiotracer (e.g., technetium-99m sulfur colloid) and/or vital dye (e.g., isosulfan blue or methylene blue) is peritumorally injected. These agents are taken up by the local lymphatic vasculature and migrate to the SLN.
3. Intraoperative localization of the SLN is performed using a gamma probe (for radiotracers) and/or visual inspection (for dye), allowing the surgeon to accurately identify the targeted node.
4. The sentinel node is surgically excised through a small incision, often along with adjacent nodes if they appear suspicious intraoperatively or are in close proximity.
5. The excised lymph nodes are then submitted to the pathology laboratory for comprehensive histopathological evaluation, including gross examination, sectioning, H&E staining, and potentially IHC analysis to assess for the presence of metastatic carcinoma.

This study specifically focuses on the fifth step, namely the laboratory processing and diagnostic evaluation of sentinel lymph nodes.

2.2 Sentinel Lymph Node Biopsy Methodologies

As discussed, SLNB encompasses a variety of procedures commonly performed in surgical pathology laboratories, each with its specific protocols, materials, and resource requirements. Given this diversity, we recognized the importance of assessing the environmental impact of SLNB as a whole. Our evaluation aimed to provide a comprehensive understanding of the ecological footprint associated with these procedures, considering not only individual techniques and their variations but also contrasting them with alternative methodologies that can achieve similar clinical outcomes.

In our assessment, we focused on both the **histological** and an alternative **molecular** methodology, which offers a clinically equivalent analysis while employing a fundamentally different methodology (Figure 1). Unlike SLNB, which is primarily rooted in histological techniques involving the physical examination of tissues, the molecular methodology leverages advanced genetic and biochemical analyses. This transition from a histological perspective to a molecular one represents a significant paradigm shift in how sentinel lymph nodes are analyzed.

By comparing the environmental impacts of SLNB with those of the alternative molecular method, we aimed to uncover the broader implications of these practices in clinical pathology. The evaluation considered various factors, including the types and quantities of materials used, energy consumption, waste generation, and potential emissions associated with each method. This comparison not only highlights the ecological considerations inherent in routine clinical practices but also serves to inform stakeholders about the potential benefits of adopting more sustainable methodologies.

2.2.1 Clinical performance

Traditionally, SLN has been carried out using histopathological examination, including H&E staining and IHC—a method known as ultrastaging. More recently, the one-step nucleic acid amplification (Molecular methodology) assay has emerged as a rapid, automated molecular alternative. Considering the Histological methodology as the golden standard multiple studies have been performed comparing the efficacy of the two methods.

A 2017 meta-analysis [56] explored 12 studies with 2833 patients, reported a pooled sensitivity of 0.87 and specificity of 0.92 for Molecular methodology, with an **area under the curve (AUC)** of 0.94. These values echoed another 2014 study [57], which also found a sensitivity of 0.87 and an even higher specificity of 0.98 for macrometastases detection. Another study [58], incorporating more recent studies (2018), reported slightly improved metrics: a sensitivity of 0.90, specificity of 0.96, and AUC of 0.98 for overall metastases; and for macrometastases, a sensitivity of 0.85, specificity of 0.98, and AUC of 0.94.

These results affirm Molecular methodology's high accuracy in detecting SLN metastases. However, sensitivity and specificity alone do not reflect real-world diagnostic implications, particularly when disease prevalence is low, as is often the case with macrometastases. In this context, **positive predictive value (PPV)** becomes critical.

Tiernan et al.[57] reported a PPV of only 0.79 for macrometastasis detection using Molecular methodology, suggesting that up to 21% of patients classified intraoperatively as having macrometastases would be overtreated undergoing axillary clearance. This discrepancy highlights a key limitation of Molecular methodology that has been also reported in other diagnostic context (Endometrial cancer)[59]

This low PPV is a direct consequence of Molecular methodology's reliance on **cytokeratin 19 (CK19)** expression as a surrogate marker for tumor size. However, CK19 expression levels do not consistently correlate with tumor volume. Notably, CK19 is absent in up to 11% of grade III tumors and 30% of triple-negative breast cancers[57].

However one of the key technical advantages of Molecular methodology is its ability to analyze the entire lymph node, thereby eliminating **tissue allocation bias (TAB)**, a known limitation of

ultrastaging which examines only thin sections and may miss focal metastases. This whole-node approach enhances Molecular methodology's sensitivity for detecting low-volume disease, including micrometastases.

Additionally, Molecular methodology delivers results within 20 to 30 minutes, making it suitable for intraoperative use and allowing immediate decisions regarding axillary management, whereas ultrastaging is more labor-intensive and typically performed postoperatively. Moreover, Molecular methodology is fully automated and yields quantitative results, minimizing the interobserver variability that often affects traditional histological evaluation. These technical advantages has been observed both in Breast Cancer and in other settings[59] [60]

2.2.2 Technical considerations

One of the most significant operational advantages of the Molecular Methodology is its rapid processing time. The assay typically delivers results within **16 to 40 minutes**, depending on the number of lymph nodes analyzed and the laboratory workflow. In contrast, the histological methodology requires **several hours to days**, due to the need for formalin fixation, paraffin embedding, serial sectioning at multiple levels, and immunohistochemical staining[60] [59]. This time differential has important clinical implications: the quick turnaround facilitates real-time intraoperative decision-making, enabling surgeons to proceed with axillary lymph node dissection immediately if metastases are detected, rather than waiting for postoperative pathology report[61]

Moreover the molecular methodology significantly reduces labor intensity by automating the lymph node analysis process, thereby minimizing the need for extensive manual handling and the specialized histopathological expertise required in the histological method[62]. This automation streamlines the workflow and allows for a more efficient allocation of human resources, as fewer and less trained personnel are needed to complete the analysis. Additionally, OSNA offers a more compact and space-efficient setup requiring only a bench-top machine instead of multiple laboratories[60] [59].

2.2.3 Histological methodology

The process typically begins with the reception and registration of the lymph node in the laboratory informatics system. This ensures proper tracking and documentation of the specimen. The lymph node is then manually cleaned using a scalpel on a plastic plate, with disposable lab-wear to maintain a sterile environment. For rapid intraoperative assessment, the node is sectioned into 5 μm slices at 2 mm intervals using a cryostat. These sections are mounted on glass slides and stained with H&E for microscopic examination. This process is typically performed in a single space dedicated to intraoperative diagnostics to expedite the analysis. The remaining tissue is then transferred to a single-use container and immersed in formalin for fixation. This process preserves the tissue structure and prevents decomposition. Fixation is typically carried out in a dedicated room with adequate ventilation to ensure the safety of laboratory personnel. Subsequently, the fixed tissue is embedded in paraffin using a dedicated tissue processor. This process facilitates the cutting of thin sections for microscopic examination. The tissue processor requires a dedicated space with appropriate ventilation to ensure proper functioning and safety. Following processing, the embedded tissue block is manually integrated into paraffin using an inclusion station in a second ventilated room. This step ensures that the tissue is properly oriented for sectioning. The tissue block is then sectioned at 3 μm thickness with a microtome. These sections are placed alternately on standard and polarized microscopy slides. Standard slides undergo automated staining and microscopic evaluation by the pathologist. Depending on the pathologist's request, approximately half of the polarized slides are subjected to immunohistochemical analysis, which involves using antibodies to detect specific proteins or molecules within the tissue, usually epithelial markers as CK19. Immunohistochemical analysis is typically performed in a dedicated laboratory with appropriate equipment and reagents. After staining, the slides are cleaned and prepared for mounting. All prepared slides are examined by the pathologist under a microscope. This process is typically performed by each pathologist in their office, although some laboratories may have dedicated microscopy rooms.

2.2.4 Molecular methodology

The molecular diagnostic process for lymph nodes typically begins with the reception and registration of the sample in the laboratory informatics system. This ensures proper tracking and documentation of the specimen. The sample is then transferred to a designated laboratory where the entire analysis will take place. This centralized approach allows for efficient workflow and quality control. Firstly, the sample is manually cleaned, as for the histological methodology, and cut into fragments not exceeding 600 mg. This step is essential to ensure that the sample is suitable for subsequent analysis. Each of these fragments is then placed into a designated plastic tube containing a stabilizing reagent(Lynorhag). This reagent helps to preserve the integrity of the nucleic acids during the subsequent processing steps. The tubes are subsequently processed in a homogenizer to ensure thorough mixing. This step is crucial for releasing the nucleic acids from the cells. From the homogenized samples, a smaller aliquot is transferred to microtubes. Here, it is mixed using an electric mixer, followed by centrifugation in a table-top mini centrifuge. This process isolates the nucleic acids from the other cellular components. The processed sample is then loaded into an analytic instrument where nucleic acids amplification is performed thought **loop-mediated isothermal amplification (LAMP)[63]** aiming at quantifying the mRNA levels of CK19. This instrument must be equipped with the sample, clean reaction cells, and a set of reagents, which includes three calibration curve standard samples, a positive and negative control, as well as primer and enzyme components.

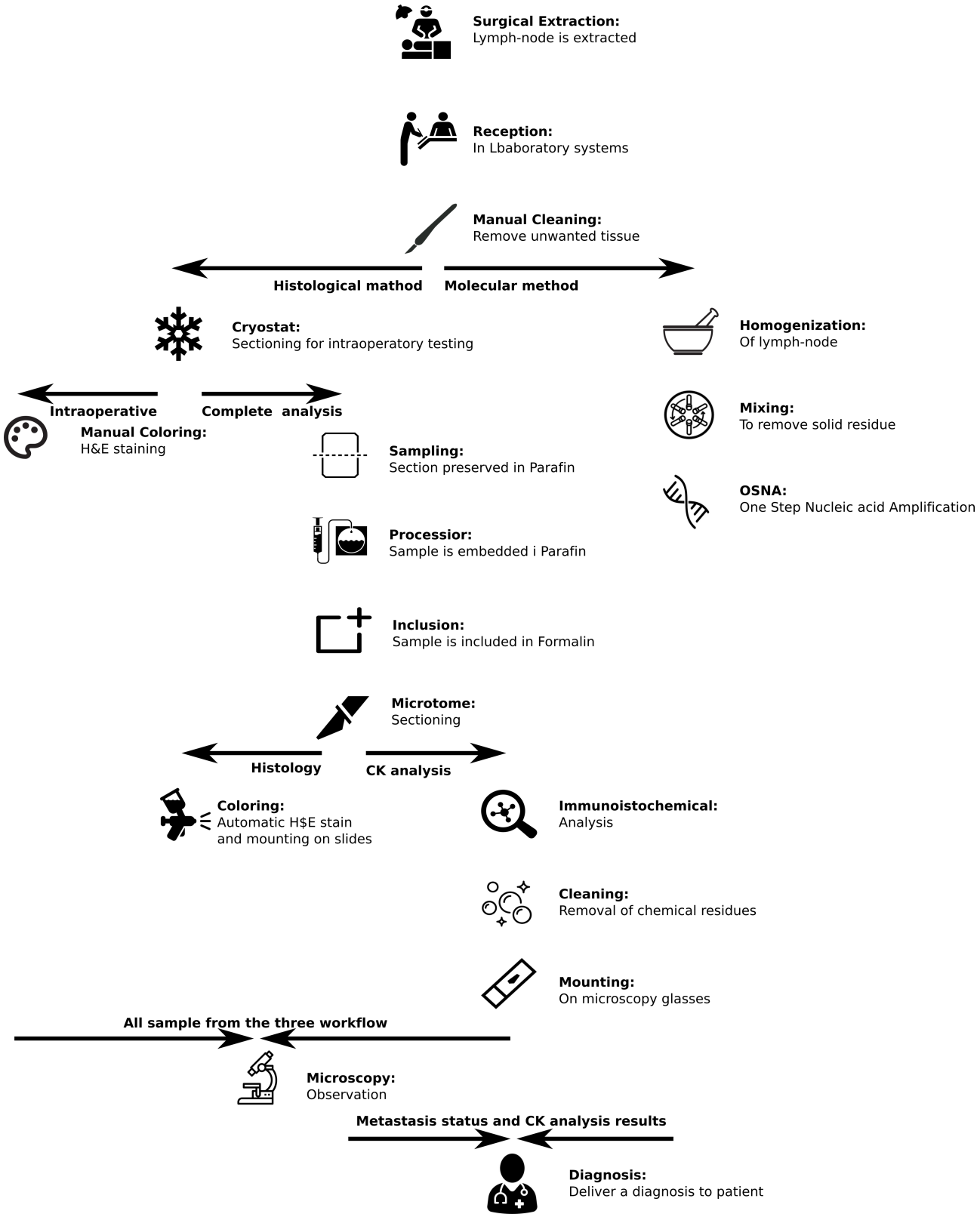

Figure 1: Histological and Molecular workflow: Graphical abstract of the two workflow from the operating room to the diagnosis.

3 Chapter : Environmental Impact Evaluation

3.1 Challenges

Environmental impact evaluation (EIA) is a critical tool for systematically assessing the potential consequences of human activities on the environment[64]. However, it is fraught with several limitations that can hinder the accuracy and effectiveness of such assessments.

One of the key challenges in EIA is the inherent complexity and interconnectedness of environmental systems. These systems are often highly dynamic and influenced by a multitude of factors, making it difficult to accurately model their behavior. This complexity can lead to uncertainties in predicting the environmental impacts of human activities[65].

Another significant limitation is the availability and quality of data. EIA often relies on incomplete or standardized data, which can introduce biases and inaccuracies into the assessment. Heterogeneous data sources and varying data quality can make it challenging to develop robust and reliable models[66].

Furthermore, the long-term effects of pollutants and other environmental stressors can be difficult to predict and assess. Many environmental impacts may take decades or even centuries to manifest, making it challenging to fully understand the consequences of human activities on the environment[67] [68].

Value judgments and stakeholder conflicts pose another challenge in EIA. Environmental impacts are often subjective and can vary depending on the values and perspectives of different stakeholders. Competing interests among stakeholders can make it difficult to reach consensus on the significance of environmental impacts. Public participation in the EIA process can also be challenging, especially in areas with diverse and sometimes conflicting interests[69].

Methodological challenges also hinder EIA. Selecting the most appropriate methods for assessing environmental impacts can be difficult, as different methods have varying strengths and weaknesses. Quantifying uncertainty in environmental impact assessments is essential but can be challenging due to the inherent variability of natural systems. Assessing the cumulative impacts of

multiple projects or activities requires sophisticated modeling techniques and data integration[70] [71].

3.2 Limitations

Even when accounting for all the above mentioned challenges that we incur while performing an EIA analysis there are some limitations that can not be directly addressed and must be remembered when interpreting the results.

Because of the heterogeneity and the vast range of contextual origins of the data required to model environmental systems, information is often collected across different spatial and temporal scales, using widely varying methods, instruments, and disciplinary frameworks. This diversity results in significant differences in data accuracy, resolution, and focus. Even when compiled into databases that appear methodologically standardized, such datasets frequently lack true comparability at the source level. Inconsistencies in definitions, metrics, and classification schemes further hinder interoperability between datasets and introduce semantic ambiguities. As a result, integrating these heterogeneous data sources into environmental models or assessments can generate substantial uncertainty, reduce the reliability of impact predictions, and complicate transparency and reproducibility. Consequently, the effectiveness of Environmental Impact Assessment is often constrained by the epistemological and technical inconsistencies embedded in the very data it relies upon[72] [73] [74] [75].

A wide range of methods have been developed to assess environmental impacts, each tailored to emphasize specific aspects of human influence on ecological systems (es. emissions, land use, biodiversity or ecosystem loss). These methods differ significantly in their scope, assumptions, and disciplinary origins, often reflecting institutional priorities and available data rather than offering a comprehensive perspective [76] [77]. This diversity highlights the fact that we are still far from fully understanding the true scope and long-term consequences of human-induced environmental change, especially in complex systems characterized by delayed feedback and cumulative effects [78]. As a result, practitioners are often required to select the methodological lens they consider most suitable for the context at hand, which inevitably introduces subjective judgments and partial representations into the assessment process [79].

3.3 Environmental Impact Evaluation Methodologies

As discussed, one of the central challenges in conducting a robust environmental impact analysis lies in developing a methodological framework capable of integrating the wide array of perspectives, disciplines, impacts involved. Given the fragmented nature of current assessment tools no single method can comprehensively capture the complexity of human–environment interactions.

To this day, a multitude of approaches has been tested and refined for environmental impact assessment. Simple methods like the **Ad-hoc approach** and the **Checklist method** are among the earliest. The *Ad-hoc approach* relies on expert judgment to identify potential impacts based on experience and intuition, offering flexibility and speed but often suffering from inconsistency and lack of transparency. The *Checklist method* uses structured lists of environmental factors to systematically identify possible impacts, providing a more organized assessment but often lacking the analytical depth. The **Matrix** method improves on this by assessing interactions between project activities and environmental factors in a more systematic way, while **Overlay Mapping** uses visual spatial data to highlight geographic impacts. More complex approaches, such as **Network Diagrams**, explore cause-effect relationships, revealing indirect and cumulative effects that simpler methods may miss. **Quantitative Models** and **Life Cycle Assessment (LCA)** take a more data-driven approach, predicting and measuring impacts in areas like air and water quality or the entire life cycle of a product, but these require significant data and expertise. **Cost-Benefit Analysis (CBA)** and **Multi-Criteria Analysis (MCA)** offer structured decision-making tools, incorporating both environmental and economic considerations, with MCA providing flexibility to weigh multiple criteria. **Environmental Risk Assessment (ERA)** focuses specifically on assessing risks posed by environmental hazards, while **Geographic Information Systems (GIS)** allow for precise spatial analysis. **Expert Judgment** taps into specialist knowledge, offering valuable insights when data is limited, though it can be subjective. Finally, **Scenario Analysis** simulates potential future impacts under different conditions, and **Environmental Indicators** simplify tracking by focusing on key metrics like carbon emissions or water quality. Each method serves specific needs depending on project scale, data availability, and complexity, making it crucial to choose the right approach for comprehensive environmental assessment[80].

4 Chapter : LCA

4.1 Definition and Standardization

LCA is defined and standardized through several key frameworks, organizations, and guidelines. The foundational standards for LCA come from the **International Organization for Standardization (ISO)**, specifically **ISO 14040 [81]** and **ISO 14044 [82]**. These standards lay out the principles, framework, requirements, and guidelines for conducting LCAs, ensuring consistency and transparency in how LCA studies are carried out globally. **ISO 14040** defines the key phases of LCA—**goal and scope** definition, **life cycle inventory analysis (LCI)**, **life cycle impact assessment (LCIA)**, and **interpretation**—while **ISO 14044** provides detailed instructions on how to implement these phases, making it the benchmark for LCA standardization.

Complementing ISO standards is the **ILCD Handbook** (International Reference Life Cycle Data System), developed by the **European Commission's Joint Research Centre (JRC) [83]**. The ILCD Handbook offers detailed guidance for conducting consistent and scientifically reliable LCAs, aligning with ISO principles. It also provides recommendations for impact assessment methods, which are crucial for practitioners, particularly in Europe. Additionally, the **Product Environmental Footprint (PEF)[84]** and **Organizational Environmental Footprint (OEF)[85]** guidelines from the European Commission aim to harmonize LCA methodologies for assessing the environmental impacts of products and organizations, promoting consistency in environmental labeling.

Beyond general environmental impacts, specific frameworks like the **GHG Protocol [86]**—developed by the **World Resources Institute (WRI)** and **World Business Council for Sustainable Development (WBCSD)**—focus on greenhouse gas emissions, providing a structured approach for life cycle assessments of product carbon footprints. Another important contribution comes from the **Society of Environmental Toxicology and Chemistry(SETAC)**, which played a foundational role

in developing LCA methodologies and continues to advance LCA practices through research, publications, and conferences.

The **UNEP/SETAC Life Cycle Initiative**, a collaboration between the **United Nations Environment Programme (UNEP)** and **SETAC**, further promotes LCA globally. This initiative focuses on developing tools and resources, such as global guidance on life cycle impact indicators, and helps extend LCA use in developing countries. For practical implementation, tools like **GaBi [87]** and **SimaPro[88]** integrate standardized LCA methodologies with extensive LCI databases. These tools follow ISO and ILCD standards and offer users access to modeling framework and impact assessment methods.

The **CML method**, developed by the **Institute of Environmental Sciences** at Leiden University, is one of the most widely used LCA methodologies, providing a set of standardized characterization factors for evaluating impacts like global warming and acidification. In North America, the **TRACI** method, developed by the **US Environmental Protection Agency (EPA)**, is commonly used, offering impact categories suited to the US context. Another important framework is **PAS 2050**, developed by the **British Standards Institution (BSI)**, which focuses on carbon footprint and complements full LCA by assessing the greenhouse gas emissions of products and services.

4.2 LCA Phases

LCA is a systematic method used to evaluate the environmental impacts of a product, process, or service throughout its entire life cycle. The LCA process is standardized through ISO 14040 and ISO 14044, which define four key phases: **Goal and Scope Definition**, **Life Cycle Inventory Analysis (LCI)**, **Life Cycle Impact Assessment (LCIA)**, and **Interpretation**. These phases ensure that the study is comprehensive, scientifically robust, and aligned with its intended purpose[89].

The first phase, *Goal and Scope Definition*, provides the foundational structure of the assessment. The *goal* specifies the study's purpose, target audience, and decision context. The *scope* defines the methodological framework, including the **functional unit:** the quantified reference against which all inputs and outputs are normalized, and the **system boundaries**, which determine the life cycle stages to be included (e.g., cradle-to-grave or cradle-to-gate).

In the **Life Cycle Inventory (LCI)**, all mass and energy flows entering and leaving the product system are quantified. This includes inputs such as raw materials, fuels, and water, as well as outputs including products, emissions to air and water, and waste. These flows are tracked across all processes within the defined system boundaries, and are typically drawn from both primary sources (e.g., direct measurements) and secondary data (e.g., databases).

LCA can be implemented using two principal methodological approaches: **Process-Based** and **Environmentally Extended Input–Output**. Process-based LCA models environmental flows using detailed unit-level data from specific industrial processes, enabling high-resolution analysis of products or systems within well-defined system boundaries. However, this approach may omit upstream or background processes due to system boundary truncation. In contrast, EEIO LCA integrates environmental extensions with national or regional economic input–output tables, capturing the full spectrum of indirect environmental impacts across entire economies. While this method offers comprehensive system coverage, it operates at a lower resolution and is less suited for product-specific assessments. Within process-based LCA, two distinct modeling paradigms are recognized: **Attributional** and **Consequential** . Attributional LCA quantifies the average environmental burdens associated with the production and consumption of a product or service under existing or historical conditions. It provides a static snapshot of current systems and is commonly used for reporting, labeling, and performance benchmarking. Conversely, Consequential LCA aims to assess the environmental implications of decisions or system changes, such as the introduction of a new product or policy intervention. This approach incorporates marginal data and employs system expansion or substitution to capture indirect effects and broader market-mediated responses, making it particularly relevant for prospective analyses and policy evaluation.

The third phase, **Life Cycle Impact Assessment (LCIA)**, translates the inventory data into potential environmental impacts. LCI flows are assigned to relevant environmental impact categories. Each flow is characterized using factors that quantify its contribution to these impacts. For example, methane has a higher global warming potential than carbon dioxide, so its impact is adjusted accordingly. Additional steps, such as normalization (comparing impacts to a reference) and weighting (assigning importance to impact categories), can be performed, but these are optional.

Finally, the **Interpretation** phase involves analyzing the results from both the LCI and LCIA to draw conclusions and provide recommendations. This phase identifies the most significant environmental impacts and may involve sensitivity or uncertainty analysis to explore how changes in key variables affect the results. The conclusions must align with the goals of the study, ensuring that the LCA provides actionable insights for improving environmental performance, such as optimizing resource use or reducing emissions. Transparency and consistency are critical in this phase, as highlighted by the ISO 14044 standard, to support informed decision-making.

4.3 Modeling Tools

Several software tools are available for conducting LCA. Leading options include **SimaPro** and **GaBi**, widely used for their comprehensive databases and features suited for research. Open-source tools like **OpenLCA[90]** and **Brightway2[91]** offer cost-effective, customizable solutions, while cloud-based platforms such as **One Click LCA[92]** and **Ecochain[93]** focus on user-friendly services. There are also specialized tools like **Umberto[94]** and **CES Selector[95]** emphasize process optimization and material selection, respectively.

However modeling a LCA always follows a structured process that integrates data collection, inventory analysis, impact assessment, and interpretation, adhering to the principles of ISO 14040 and ISO 14044.

In this case we modeled our systems on SimaPro being one of the most reliable and widely used LCA software.

4.4 Databases

Several specialized databases have been developed to support LCA by providing quantified LCI data, enabling consistent modeling of indirect data across various sectors. Among the most widely used is the ecoinvent database, maintained by the ecoinvent Centre in Switzerland. It offers a comprehensive, peer-reviewed dataset with global coverage and particularly strong European representation, supporting multiple system models such as cut-off and allocation at the point of substitution. Another major source is the **GaBi** database, developed by Sphera (formerly Thinkstep), which is widely used in industrial applications and offers region-specific, proprietary datasets with particular strength in European and North American manufacturing sectors. The **U.S. Life Cycle Inventory** Database, developed by the **National Renewable Energy Laboratory (NREL)**, provides free, publicly accessible data tailored to U.S. industrial processes, and is frequently used in government and academic research.

In the European context, the **European Reference Life Cycle Database (ELCD)**, maintained by the European Commission's Joint Research Centre, offers datasets aligned with EU environmental policy and regulatory frameworks. For studies focused on agricultural and food systems, **Agri-footprint**, developed by Blonk Consultants, delivers high-resolution inventory data covering crop cultivation, livestock production, and food processing. For macro-level environmental modeling, particularly in EEIO assessments, **Exiobase** provides a global, multi-regional input–output framework linking environmental pressures to economic activities across over 40 countries. Lastly, for region-specific assessments in East Asia, the **Inventory Database for Environmental Analysis (IDEA)** database, developed by the Japan **Environmental Management Association for Industry (JEMAI)**, offers detailed process data tailored to Japanese industrial systems.

For consistency and reproducibility sake most of the data in this study has been derived from ecoinvent.

4.5 Impact Assessment Methods

A variety of LCIA methods have been developed to characterize and quantify the environmental impacts derived from inventory flows in LCA. These methods differ in geographic focus, impact categories, modeling depth, and the degree of normalization and weighting applied.

One of the earliest and most standardized approaches is the **CML Baseline method**, developed at the Institute of Environmental Sciences at Leiden University in the Netherlands. CML is a midpoint-focused method that provides scientifically robust and transparent characterization factors for categories such as global warming potential, acidification, eutrophication, ozone depletion, and photochemical ozone creation. Its strength lies in its avoidance of value-laden assumptions and its widespread compatibility with ISO standards, making it a favored method for comparative studies[96].

In the United States, the **Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts (TRACI)** was developed by the U.S. Environmental Protection Agency to provide impact assessment factors relevant to North American environmental conditions and regulatory frameworks. TRACI includes categories such as global warming, acidification, eutrophication, smog formation, and human health impacts. Its alignment with U.S. policy and data sources has made it the standard for LCA studies in that region[97].

A more integrative approach is embodied in the **ReCiPe method**, a collaborative development by Dutch institutions including RIVM, Radboud University, and PRé Consultants. ReCiPe merges the midpoint modeling strength of CML with the endpoint structure of Eco-Indicator 99, offering a unified framework that includes 18 midpoint categories and three endpoint indicators (human health, ecosystem quality, and resource depletion). It provides analysts with the flexibility to select from three cultural perspectives—Hierarchist, Egalitarian, and Individualist—depending on assumptions about time horizon and risk aversion[98].

Similarly comprehensive is **IMPACT 2002+**, developed at the Swiss Federal Institute of Technology in Lausanne. This method combines midpoint and endpoint modeling, placing particular emphasis on human toxicity, ecotoxicity, resource extraction, and other damage-related

categories. It is especially valued for its nuanced treatment of toxicological impacts, which are often underrepresented in simpler models[99].

Eco-Indicator 99, also developed by PRé Consultants, takes an exclusively endpoint-focused approach. It condenses environmental impacts into three damage categories—human health, ecosystem quality, and resources—making it well-suited for product design, labeling, and simplified decision-support in industry. Although it lacks the transparency of midpoint-based approaches, its simplicity and ease of interpretation have contributed to its popularity, particularly in European eco-design contexts[100].

To address the need for standardized and policy-aligned LCIA methods in Europe, the European Commission developed the **International Reference Life Cycle Data System (ILCD)** method through its Joint Research Centre. ILCD uses a midpoint approach with a wide range of scientifically validated impact categories, including climate change, human toxicity, particulate matter formation, ionizing radiation, and resource depletion. Its rigorous methodological foundations and compatibility with European regulation make it one of the most widely adopted frameworks for LCA studies conducted within or for the European Union[101].

A distinctive approach is offered by the **Environmental Priority Strategies (EPS)** method, originating in Sweden. EPS translates environmental impacts into monetary values that represent the estimated cost of preventing damage to human health, biodiversity, ecosystem services, and resource stocks. This economic valuation makes EPS particularly useful for product development and eco-design, where cost–benefit considerations are integral to the design process[102].

Finally, the **Environmental Footprint (EF) method** was introduced as part of the European Commission's PEF initiative. The EF method aims to harmonize environmental impact assessment across industries and sectors in the EU. It includes midpoint categories such as climate change, water scarcity, land use, ecotoxicity, and resource use, and is designed to support comparative environmental claims and regulatory compliance[103].

The CML method was selected due to its transparent, midpoint-focused approach, which allows for a scientifically robust and value-neutral comparison across multiple impact categories. By focusing on midpoint indicators CML enables a detailed characterization of environmental burdens without aggregating results into potentially subjective endpoint values. This methodological clarity is

particularly advantageous when comparing functionally similar but procedurally diverse diagnostic workflows, as it ensures that variations in impact are attributable to specific life cycle stages or processes rather than to weighting assumptions. Additionally, the widespread acceptance and compatibility of CML with international LCA standards further supports its selection as an appropriate and reliable tool for comparative environmental evaluation in the healthcare context.

4.6 Chemicals

One of the key challenges encountered stems from the wide variety of compounds present in the reagents used in the diagnostic methods under consideration. Only a small portion of these compounds are cataloged in commercially available databases. Additionally, when such data is available, it often pertains to industrial-grade rather than pharmacological-grade compounds. Assessing the environmental impact of pharmaceuticals is further complicated by the confidentiality surrounding their manufacturing processes. While several methodologies have been proposed for this purpose [104], the proprietary nature of many compounds restricts access to detailed information.

To ensure methodological reproducibility and consistency, a practical approach was adopted. Two proxy chemicals were selected—one to represent **organic** compounds and one for **inorganic** compounds—based on the average composition of the 20 most commonly used chemicals[105]. These proxy chemicals were then used to account for the organic and inorganic components of the reagents, with any unspecified substances assumed to be ultrapure water, a standard solvent.

4.6.1 Database

The data for the proxy chemicals and their composition were sourced from the ecoinvent[106] database. The choice of ecoinvent ensured that the proxy chemicals selected were backed by comprehensive and reliable environmental data, facilitating more accurate modeling of the organic and inorganic components. This database offers robust datasets, covering a wide array of industries, which allows for greater transparency in evaluating the environmental footprint of both organic and inorganic reagents. By leveraging ecoinvent's extensive resources, we were able to enhance the reliability and accessibility of our assumptions regarding chemical composition, thus contributing to the overall reproducibility and consistency of the study.

4.6.2 Chemicals categories

In the context of the systems under study, and considering the proxy chemical approach adopted for modeling, three distinct categories of chemical reagents can be identified. The **first category**, referred to as *industrial-grade chemicals*, corresponds to substances with characteristics aligned with those represented by the ecoinvent proxy chemical dataset. These reagents typically exhibit lower purity levels and are widely used in histological protocols, with the exception of certain substances required for immunohistochemical analysis. The **second category**, designated as *medical-grade* or *fine chemicals*, includes high-purity or structurally complex compounds. These are predominantly employed in molecular diagnostic methodologies and immunohistochemical assays, where greater chemical specificity and production rigor are required. The **third category** pertains to reagents used in LAMP technology, which contain synthetic **ribonucleic acid (RNA)**. Due to the intricate synthesis processes and the presence of only trace amounts of RNA in the final formulations, these reagents cannot be appropriately classified under either of the first two categories. Instead, they represent a distinct class that necessitates a separate treatment within the environmental modeling framework, owing to the unique challenges associated with RNA production. Based on these distinctions a different method was applied to each category.

4.6.2.1 Industrial

The first category of reagents evaluated includes those classified as "Industrial grade," which typically have a purity level below 95%. These reagents are commonly used in industrial processes where such high purity is not a requirement. One key advantage of this category is that the composition of industrial-grade reagents is usually declared, providing detailed information on their constituent chemicals. As a result, it was possible to model the fractional composition of organic, inorganic, and solvent components with a high degree of reliability.

This transparency in composition allowed us to accurately quantify the proportions of organic and inorganic compounds, as well as the solvent content, ensuring a more precise and reproducible model.

4.6.2.2 Medical

For the second grade of reagents, a "fine chemicals" **multiplier of 25x** was applied, as recommended by Wernet et al. in 2010[107]. This multiplier reflects the more stringent production standards and higher resource intensity involved in the manufacture of fine chemicals, such as those

used in pharmaceutical synthesis. The 25x factor was calculated in the cited paper to account for the increased energy, raw materials, and environmental impacts associated with producing these chemicals, compared to simpler industrial-grade compounds. This adjustment ensures that the LCA more accurately represents the environmental burden of these high-purity substances.

4.6.2.3 RNA

The third grade of reagents includes synthetic RNA oligonucleotides, which poses significant challenges due to the complexity of its synthesis. The production of synthetic RNA involves multiple stages, often requiring manual work by technicians across various production pipelines. This intricate process includes steps such as nucleotide coupling, purification, and quality control, each contributing to the overall complexity.

Due to this inherent complexity, a direct evaluation of the environmental and resource impacts associated with synthetic RNA synthesis was not feasible. To address this, the same "fine chemicals" multiplier used for second-grade reagents was applied, recognizing that the resource-intensive nature of RNA production is similar to other fine chemicals. Additionally, a **sensitivity analysis** was conducted to evaluate the robustness of the multiplier itself, ensuring that variations in the estimation would still provide reliable insights into the environmental impact of these advanced reagents. This approach allowed for a more nuanced assessment while accounting for the uncertainties inherent in modeling such a specialized synthesis process.

4.7 System Efficiency

4.7.1 Source of variation

In SLNB procedures, time is a critical factor due to the clinical nature of the process. These procedures are typically performed on demand, meaning they must be carried out promptly when required, without the opportunity to pool samples to increase efficiency. SLNB is often employed as an intraoperative analysis, where the sentinel lymph node is extracted during surgery, and immediate results are necessary to guide the surgical decision-making process. The patient remains in the operating room while awaiting the outcome, which influences whether further surgical intervention is required.

For histological methodology, the intraoperative component is limited to cryostat sectioning, staining, and microscopic examination. These steps are performed rapidly to provide immediate feedback to the surgical team. However, the remaining parts of the histological analysis, which may involve more detailed tissue evaluation, are performed later and do not carry the same urgent time constraints.

In contrast, molecular methodologies for SLNB allow for the entire analysis to be completed intraoperatively due to the short time required for molecular assays. This rapid turnaround makes it possible to deliver a complete diagnosis during surgery,

Additionally, the machines employed in SLNB and other diagnostic procedures differ significantly in their operational capacities and technical configurations. These instruments are typically used across a range of analytical workflows throughout the day, contributing to highly variable utilization patterns.

Importantly, the capacity mismatch between different machines often necessitates multiple operational cycles of one device to fully process the output generated by another or vice versa, thereby requiring staggered or repeated runs to complete the analysis. Furthermore, machines may be operated at full capacity or underloaded with only a few samples, depending on daily diagnostic

demand and scheduling. This variation in usage has a direct impact on the overall energy and resource efficiency of the diagnostic process, as underutilization leads to higher per-sample environmental burdens.

Consequently, the overall process efficiency is influenced not only by the number and complexity of tests performed but also by the degree to which machine capacities are optimally aligned and coordinated across the diagnostic workflow.

Finally, many procedures, particularly within histological methodologies, are performed manually by technicians, which introduces an additional layer of variability in efficiency. The skill, experience and habits of individual technicians can significantly impact the speed and efficiency of the procedures.

4.7.2 Scenario analysis

To address potential variations in workflow efficiency, the analysis included two distinct scenarios for each diagnostic approach: maximum efficiency and minimum efficiency. These scenarios represent the extreme limits of possible efficiency outcomes, serving as useful boundaries to frame the true efficiency that can fluctuate significantly on a day-to-day basis.

By defining these limit cases, the analysis can better illustrate the range of operational efficiency that may occur in a real-world laboratory setting. **Maximum efficiency** represents an ideal scenario where all processes are performed with optimal conditions with all the machines running at full capacity and minimal resources waste reflecting a highly streamlined workflow. In contrast, **minimum efficiency** captures the potential slowdowns and inefficiencies that may arise due to various factors, such as equipment working at suboptimal capacity, technician derived inefficiency or lack of resources optimization.

These two scenarios enable a more comprehensive understanding of the operational dynamics within diagnostic laboratories[108]. They also highlight the inherent variability in efficiency that can impact diagnostic turnaround times and, ultimately, patient care.

4.8 Building Based Evaluation

Taking into account the modularity of the hospital building for the evaluation of the environmental conditions required for laboratory procedures, we adopted a space-based approach in the analysis. Each diagnostic process was assigned to a specific room according to its environmental requirements, and the allocation was based on the duration of the procedure. Importantly, the environmental burden of the building's systems was attributed to each diagnostic process only for the time it was actually in use, rather than over the total operational time of the environmental systems. As a result, the analysis reflects the environmental impact of a single FU diagnostic process without including idle or unused system time. This was done to have a more precise evaluation of the single diagnostic procedure undependable from the throughput of the hospital.

4.9 Uncertainty Analysis

Uncertainty analysis focuses on identifying, quantifying, and evaluating the uncertainties inherent in data inputs, modeling assumptions, and methodologies employed in the LCA process. Various sources contribute to uncertainty, including data variability, measurement errors, and gaps in available information. The primary goals of conducting an uncertainty analysis are to assess the reliability of the data and to provide us a better perspective during results interpretation.

The analysis is based on the application of a pedigree matrix, which is used to assign uncertainty scores to each input parameter in the model. These scores reflect the data quality.

Each pedigree score is then translated into an estimate of the geometric standard deviation , under the assumption that the uncertainty in the input data follows a log-normal distribution. This allows for modeling each input parameter as a probabilistic variable characterized by a geometric mean and a corresponding geometric standard deviation derived from the pedigree assessment.

Subsequently, a **Monte Carlo simulation** is performed with multiple iterations, where values for each input parameter are randomly sampled from their respective log-normal distributions. This stochastic sampling propagates input uncertainties through the model, allowing us to evaluate their

influence on the output. The result is a probabilistic distribution of outcomes, which provides insights into the robustness and sensitivity of the model results to input uncertainty.

We employed a **five-hundred runs Monte Carlo simulation** for each of the four model.

4.9.1 Pedigree matrix

The pedigree matrix is a tool used in LCA to assess the quality and uncertainty of data, particularly in cases where direct measurement errors are not available. It was first introduced by B. Weidema in 1996[109] to help quantify uncertainty in data derived from various sources. The pedigree matrix evaluates the uncertainty of data through qualitative judgments, based on a set of defined criteria. Each criterion assesses a different aspect of the data's reliability, assigning scores that collectively provide a more comprehensive understanding of uncertainty.

The pedigree matrix breaks down uncertainty into multiple dimensions or criteria, with each dimension assessing a specific quality aspect of the data. Typically, the following five dimensions are used:

1. **Reliability of the Data Source**: This criterion assesses the trustworthiness of the data source. It evaluates whether the data come from direct measurements, expert estimates, or less reliable sources like assumptions or extrapolations. More reliable sources, such as experimental or industry-reported data, receive better scores, while less reliable sources, like secondary literature, receive lower scores.
2. **Completeness of Data**: This dimension focuses on how well the data cover the specific aspect under analysis. For example, it evaluates whether the data represent the entire system or just a portion of it. Incomplete data, or those missing significant elements, increase uncertainty and therefore receive a lower score in this category.
3. **Temporal Correlation**: Temporal correlation refers to how well the data match the time frame of the process being analyzed. Data collected close to the time period of the study receive a high score, while older or outdated data are given a lower score because of the risk of reduced accuracy over time, especially in dynamic systems.

4. **Geographical Correlation**: This criterion assesses how well the data correspond to the geographical region of the system under study. If data were collected from the same region, they receive a high score, while data from other regions may be less representative and are given lower scores due to potential differences in environmental conditions, resource availability, and other location-specific factors.
5. **Technological Correlation**: Technological correlation looks at the match between the technology described in the data and the technology in the system being assessed. If the data accurately reflect the technology used in the process or system, the score will be high. Data based on outdated or different technologies will score lower, as they may not capture the specific characteristics or efficiencies of the current system.

4.9.2 Monte Carlo simulations

Monte Carlo simulation is a computational technique that uses random sampling to estimate mathematical or physical systems that are difficult or impossible to solve analytically. At its core, the method involves generating a large number of random inputs to simulate a model of a system or process and then analyzing the distribution of outcomes. The simulation repeatedly samples values from specified probability distributions (e.g. log-normal) for input variables and computes corresponding outcomes through a deterministic function. Over many iterations the results converge to a statistical distribution of the possible outcomes, allowing for estimation of metrics such as expected values, variances, and confidence intervals[110].

In LCA, Monte Carlo simulation is used to propagate uncertainty from input data through to the final impact results, and therefore determine confidence intervals[111].

4.10 Sensitivity Analyses

Sensitivity analysis is a quantitative method used to determine how variations in input parameters of a model influence its output, providing critical insight into the robustness and reliability of model predictions. It allows researchers to identify which inputs have the most significant impact on the results, thereby prioritizing data collection and refining model accuracy. There are two main types: local sensitivity analysis, which assesses the effect of small changes in one input at a time, and global sensitivity analysis, which evaluates the combined effects of variations across all inputs[112].

In this context a local sensitivity analysis was performed on the RNA reagents multiplier to asses the overall effect of the complexity of the RNA synthesis on the systems impact.

4.10.1 Linear regression

We employed a linear regression-based sensitivity analysis to evaluate the uncertainties associated with the impact of RNA-based reagents. To do this, we systematically varied the RNA reagents multiplier applied to the estimated impact of the reagents across a defined range and ran the same model for each variation. The resulting set of model outputs, corresponding to different multiplier values, was then used to fit a linear regression model. In this setup, the multiplier served as the independent variable, and the overall system impact as the dependent variable. The regression coefficients provided a direct measure of how changes in the input parameter influenced the model output, indicating both the direction and magnitude of sensitivity[113].

Linear regression is particularly beneficial in this context because it provides a clear and simple framework for understanding the degree of sensitivity of the outcomes to changes in input variables .

Section 2 : Analysis

5 Chapter : Goal and scope

5.1 Goal

The objective of this LCA is to evaluate the environmental impact associated with the SLN diagnostic procedure, a widely utilized and methodologically complex process in surgical pathology. Due to its integration of multiple analytical techniques commonly employed across diverse diagnostic workflows, SLN diagnostics serves as a representative model for assessing the broader environmental footprint of pathology laboratory operations. In addition to evaluating the standard diagnostic pathway, the study also includes a novel molecular approach to investigate the potential environmental implications of integrating emerging technologies into routine practice. This dual focus provides a foundation for understanding both the current and future environmental burdens of surgical pathology.

5.2 Scope

5.2.1 Functional unit and Declared unit

LCA necessitates the establishment of a **functional unit (FU).** This standardized unit serves as a reference point for comparing the environmental performance of different options. In the context of SLNB, however, the biological variability of lymph nodes presents a unique challenge. These nodes can vary significantly in size, composition, and other characteristics, making it difficult to define a fixed and representative functional unit. In some cases, analyzing a single lymph node may not be sufficient to reach a diagnosis, while in others, particularly large nodes may require multiple diagnostic procedures to be fully examined. Consequently, a single lymph node may not consistently represent a complete functional unit for the purposes of analysis.

To overcome this obstacle, the concept of a **Declared Unit (DU)** can be employed. A Declared Unit is a standardized unit of measurement that is used for reporting LCA results, regardless of the actual unit of analysis processed by the LCA model. By adopting this approach, it becomes possible to establish a consistent and comparable basis for evaluating the environmental impacts associated with SLNB procedures.

The Declared Unit allows for the normalization of LCA results, ensuring that the environmental performance of different SLNB procedures can be accurately compared, even when they involve lymph nodes of varying dimensions and characteristics.

5.2.1.1 Declared Unit definition

We defined a DU that encompasses the **complete analysis of a single, median-sized SLN for metastasis assessment and CK19 analysis**. . This specification provides a standardized reference point for evaluating the environmental impacts associated with the analysis of lymph nodes in clinical settings.

5.2.1.2 Sentinel lymph-node heterogeneity

Sentinel lymph nodes are inherently non-uniform in size and composition,this variability is further amplified in the SLNs considered in this instance, as they are frequently affected by tumor growth. Tumor involvement can cause significant alterations in the size, shape, and internal structure of the SLN, making their dimensions even more inconsistent.

Additionally, the reporting of the characteristics of the removed SLNs occurs during surgery, where precise measurement is often challenging. This can lead to some variability in the recorded measurements, which may not always reflect the true dimensions or composition of the SLN. Factors such as tissue handling, swelling, or the inherent difficulty in measuring irregularly shaped biological structures contribute to potential inaccuracies in these initial observations.

Thus, the natural variability of SLNs, compounded by tumor involvement and the challenges of intraoperative documentation, results in a broad range of reported SLN characteristics. This makes standardization difficult and introduces potential discrepancies that must be accounted for during analysis.

In the context of this study, two critical characteristics of the SLN play a significant role in determining the definition of the functional unit: its length along the major axis and its weight. These factors are particularly important because they affect how the SLN is processed in different methodological approaches—histological and molecular.

For the histological methodology, the SLN must be sectioned into thin slices along its longest axis to ensure accurate examination of the tissue structure under a microscope. This process is essential for identifying key histopathological features and ensuring that the entire SLN is adequately analyzed. Therefore, the length of the SLN on its major axis is a crucial factor in determining how many slices can be obtained and how well the tissue can be examined, consequently the number of obtained slices will directly impact the amount of resources needed for the analytical procedures.

On the other hand, in the molecular methodology, the physical size of the SLN is less relevant because the molecular analysis does not require thin sectioning. Instead, the focus shifts to the weight of the sample, as the equipment used for molecular testing has a strict weight limit. Specifically, the machine used in this study can only accommodate samples weighing up to 600 mg.

These two distinct requirements—the need for thin slicing in histology based on length and the weight constraint in molecular analysis—highlight the different ways in which the SLN must be handled depending on the methodology being used. Consequently, we had to standardize the declared unit based on both the characteristics.

5.2.1.3 Methods – median Lymph-node

The analysis focused on SLNs processed at our hospital over a two-year period, yielding an initial dataset of approximately **600 samples**. In the first step of data cleaning, we removed all samples with incomplete or missing information.

We then investigated the relationship between the weight and length of the SLNs, as these two characteristics are central to the definition of a declared unit. A direct relationship was identified between weight and length, prompting us to employ the **weight-to-length ratio (W/L ratio)** as a standardization measurement for the subsequent step.

To improve the reliability of the dataset, we examined the distribution of the W/L ratio and identified any outliers. All samples with W/L ratios falling outside 1.5 standard deviations from the mean were excluded, as these outliers could skew the overall analysis (Figure 2).

Following these data-cleaning steps, the final dataset comprised around **400 samples**. With this refined set, we performed statistical analyses, calculating both the median length and weight.

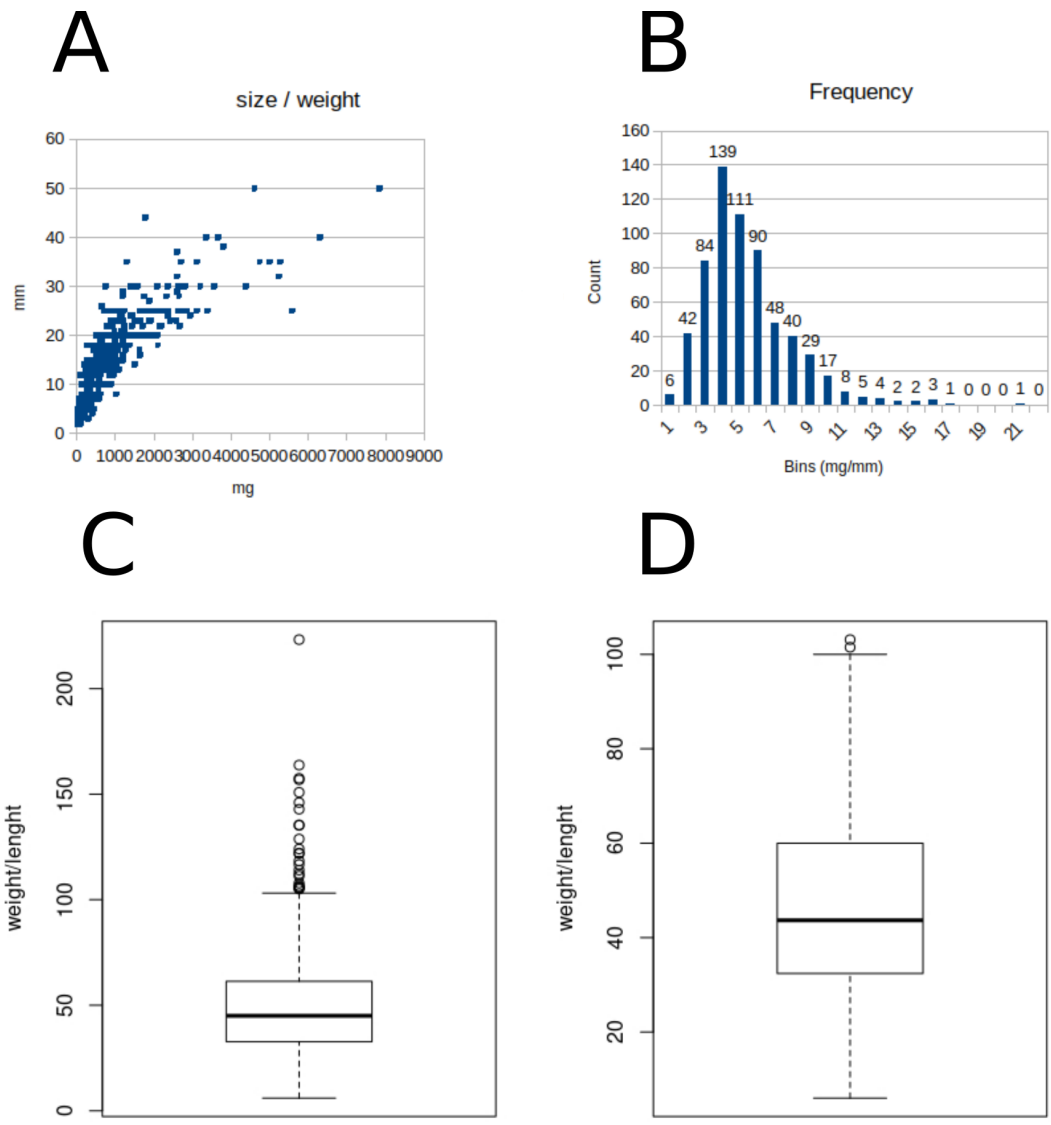

Figure 2: Median Lymph-node - Panel A: Relationship between lenght and weight. Panel B & C: Frequency distribution on the W/L ratio with outliers. Panel D: W/L ratio distribution without outliers

5.2.1.4 Results – median Lymph-node

The analysis identified a median weight-to-length (W/L) ratio of 45 mg/mm, corresponding to a median SLN length of approximately 13 mm and a median weight of around 550 mg. These characteristics suggest that, during the histological process, the median SLN would need to be sectioned into 7 parts for intraoperative analysis and approximately 60 slices for the complete histological examination (ultrastaging). Meanwhile, for the molecular methodology, the median SLN can be processed as a single sample without further sectioning.

5.2.1.5 Study setting

The analysis was conducted within the clinical pathology laboratory of the **IRCCS - Azienda Ospedaliero-Universitaria of Bologna**, an institution recognized as an **Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS)**. This designation means the hospital combines clinical care with scientific research, focusing on improving healthcare while advancing medical knowledge. The analysis reflects the status of the systems as they were in 2022-2023.

The IRCCS consists of multiple pavilions, with Pavilion 18 serving as the primary focus of this study due to its concentration of clinical pathology laboratories. This pavilion houses a variety of departments beyond just the clinical labs, including classrooms, a mortuary, and additional laboratory spaces that support various hospital functions.

The clinical pathology laboratories occupy a significant portion of the -1 floor, with additional rooms allocated on ground floor and 1st floor, as shown in Figure 3, 4, 5

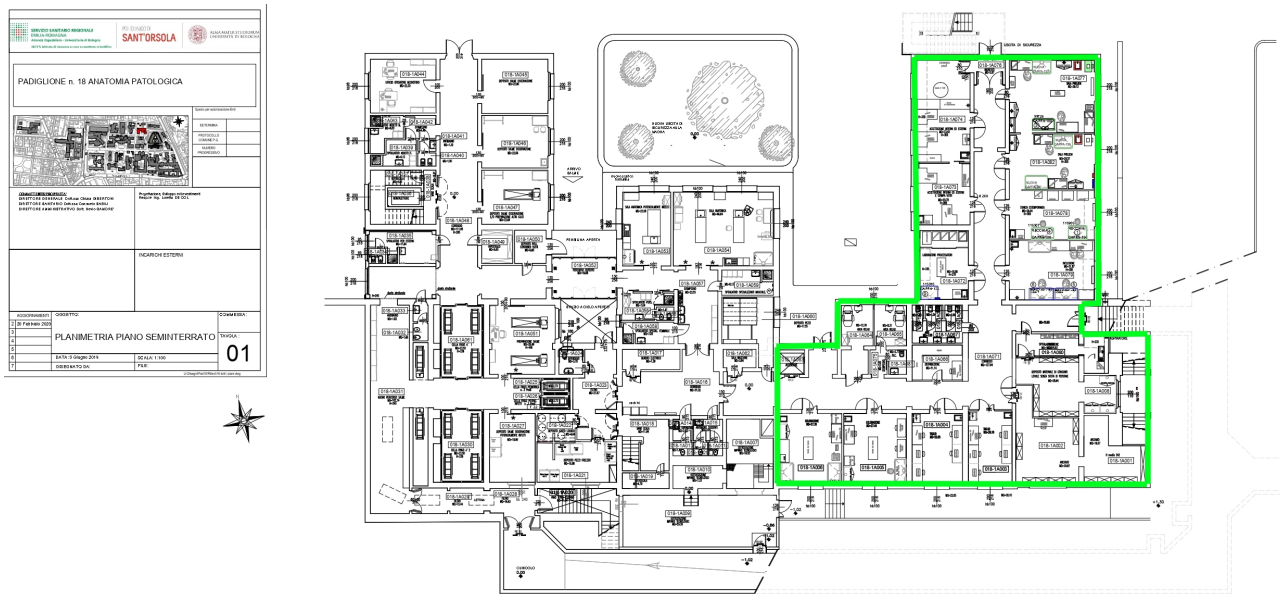

Figure 3: Basement floor, encircled in green are the areas occupied by the interested laboratories.

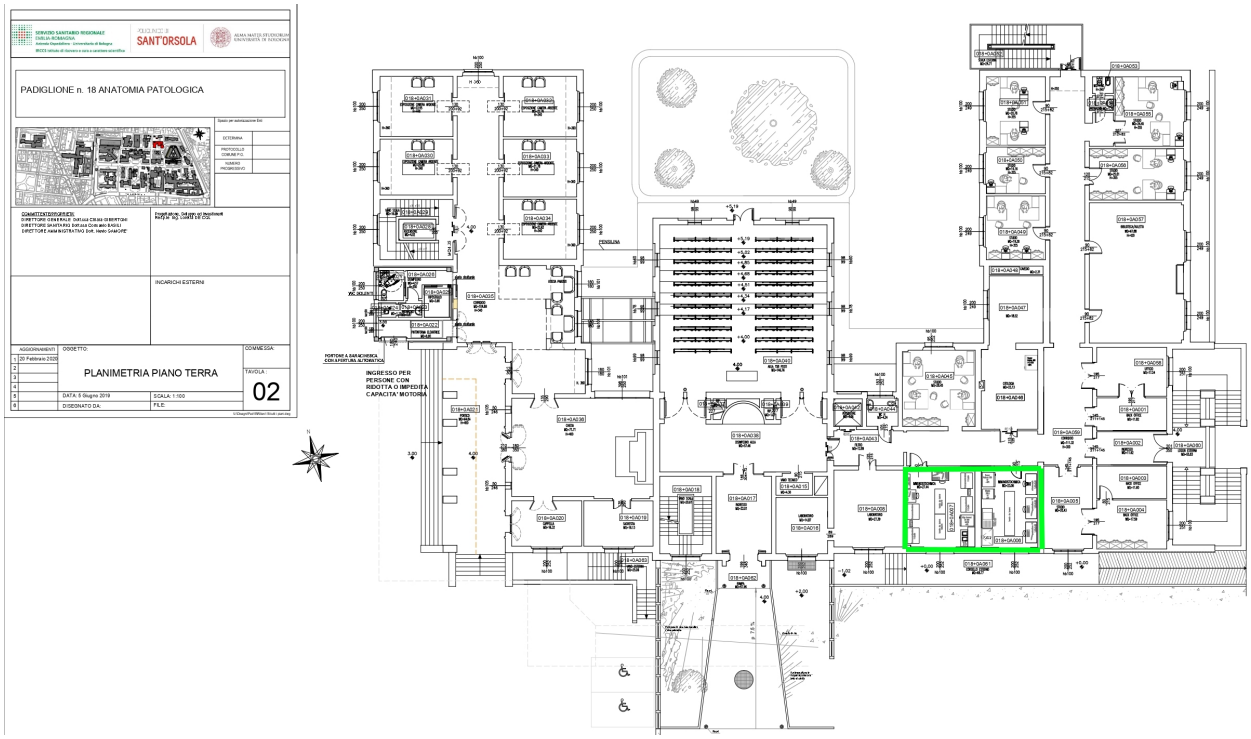

Figure 4: Ground floor, encircled in green are the areas occupied by the interested laboratories.

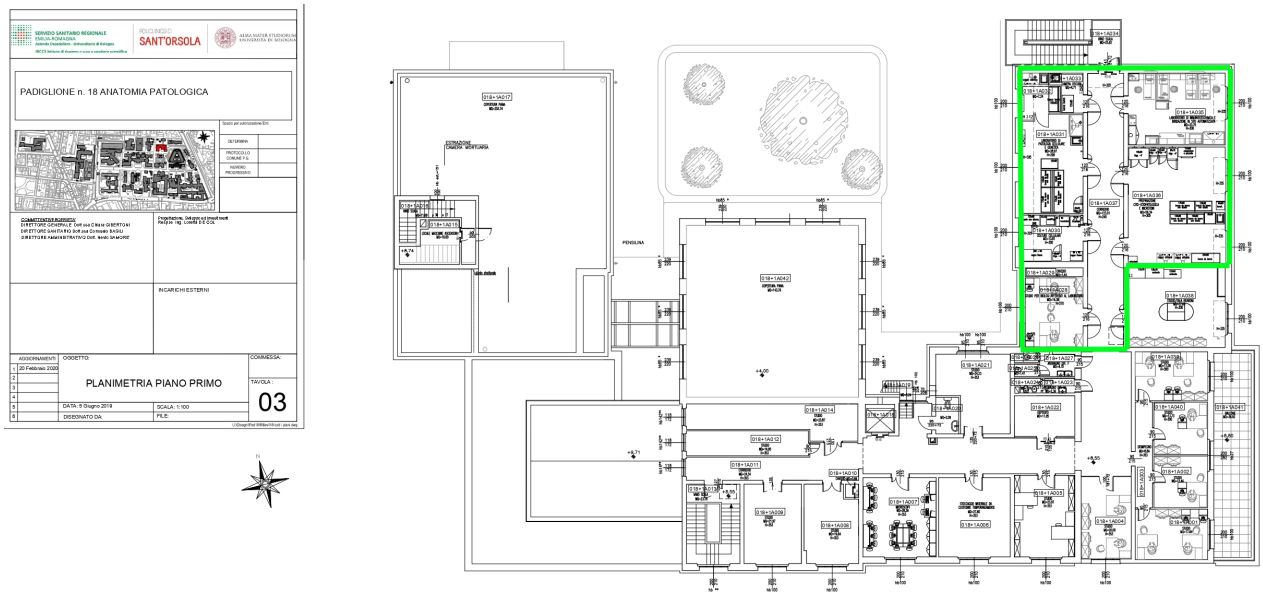

Figure 5: First floor, encircled in green are the areas occupied by the interested laboratories.

5.2.2 System boundaries

5.2.2.1 Definition

System boundaries refer to the limits that define what processes, activities, and life cycle stages are included in the analysis and which ones are excluded. Essentially, the system boundaries determine the scope of the LCA by outlining the specific aspects of a product's life cycle that will be assessed, from raw material extraction to disposal or recycling, depending on the study's goal.

This analysis applied a "cradle to grave" prospective meaning that encompasses the entire life cycle of a product, starting from the extraction of raw materials (the "cradle") and extending to the disposal or end-of-life treatment (the "grave").

The key stages covered in a cradle-to-grave assessment include raw material acquisition, manufacturing and production, transportation, the use phase, and end-of-life disposal or recycling [114].

5.2.2.2 Building systems

Given that the analysis focuses on examining all the processes necessary to perform SLNB, excluding long-term requirements, we initially aimed to include both the lighting and HVAC (Heating, Ventilation, and Air Conditioning) systems required for the laboratory spaces in the models.

After a thorough evaluation of space utilization and system requirements, it was determined that incorporating the laboratory's lighting system into the model was feasible. In contrast, the HVAC system was deliberately excluded from the model.

In the preliminary iterations of the modeling we aimed to include both the centralized heating system and the air conditioning present in some the laboratory considered. To this aim we investigated the systems layout of the facility and concluded that was not feasible to obtain a reliable evaluation of their impact without introducing extremely imprecise and inconsistent data in the models. This variability in the HVAC data is due to a variety of causes:

The hospital relies on a **single centralized heating facility** to manage the heating needs across its entire complex, which includes 23 medical pavilions along with numerous administrative and support buildings. Over the years, the hospital has expanded organically, and as a result, the heating system layout does not follow a uniform, building-by-building structure.

This decentralized growth has led to several challenges in system management and data monitoring. First, there are no individual monitoring stations for each building, which makes it difficult to gather specific consumption data per structure. Additionally, certain buildings are supplied by **multiple, independent branches** of the centralized heating network. This branching further complicates the system layout, as it disperses heating supply lines across various zones without identifiable nodes or control points that could serve as clear measurement locations.

Due to these factors, isolating the heating demand or energy consumption for each building or operational area is currently impractical. The lack of building-specific monitoring data and the complexity of the system layout hinder efforts to track and analyze heating usage with precision. Consequently, incorporating heating data into the facility's overall energy model would introduce a high level of inaccuracy, as specific contributions from each area cannot be easily evaluated.

When evaluating the air conditioning system, two significant limitations emerged that prevented a reliable assessment.

Firstly, similar to the issues encountered with the heating systems, there was no predefined development plan for the HVAC setup. As a result, the investigated building is served by three separate air conditioning systems, each of which also supplies nearby buildings. This arrangement lacks any dedicated monitoring stations on the various branches, making it difficult to track or measure specific data for this building alone.

Secondly, each room is equipped with a manual control panel for the system, allowing occupants to adjust settings individually. This manual control leads to irregular usage patterns depending on who is operating the system at any given time, resulting in inconsistent performance across different rooms and areas.

Additionally, the option for personnel to open windows manually in various laboratories further complicates matters. This combination of manual adjustments, absence of monitoring, and open-window usage prevented us from conducting a sufficiently precise evaluation of the HVAC systems' performance.

5.2.2.3 Results – system boundaries

The system boundaries, as illustrated in Figures 7 and 6, encompass all processes directly carried out within the laboratory, along with the processes involved in the manufacturing and delivery of tools, consumables, and reagents required for operations. Additionally, the model includes the disposal of waste generated during laboratory activities, whether classified as hazardous or recyclable. Energy consumption is also accounted for, including both the energy used by the laboratory equipment and the energy required to illuminate the rooms.

However, several elements are deliberately excluded from the model. These exclusions include the construction and maintenance of both the laboratory's physical infrastructure and its machinery, the HVAC system, and the surgical procedure of lymph node extraction. The rationale for these exclusions is that they either fall outside the immediate operational scope of the laboratory or involve complex variables that are not measurable consistently.

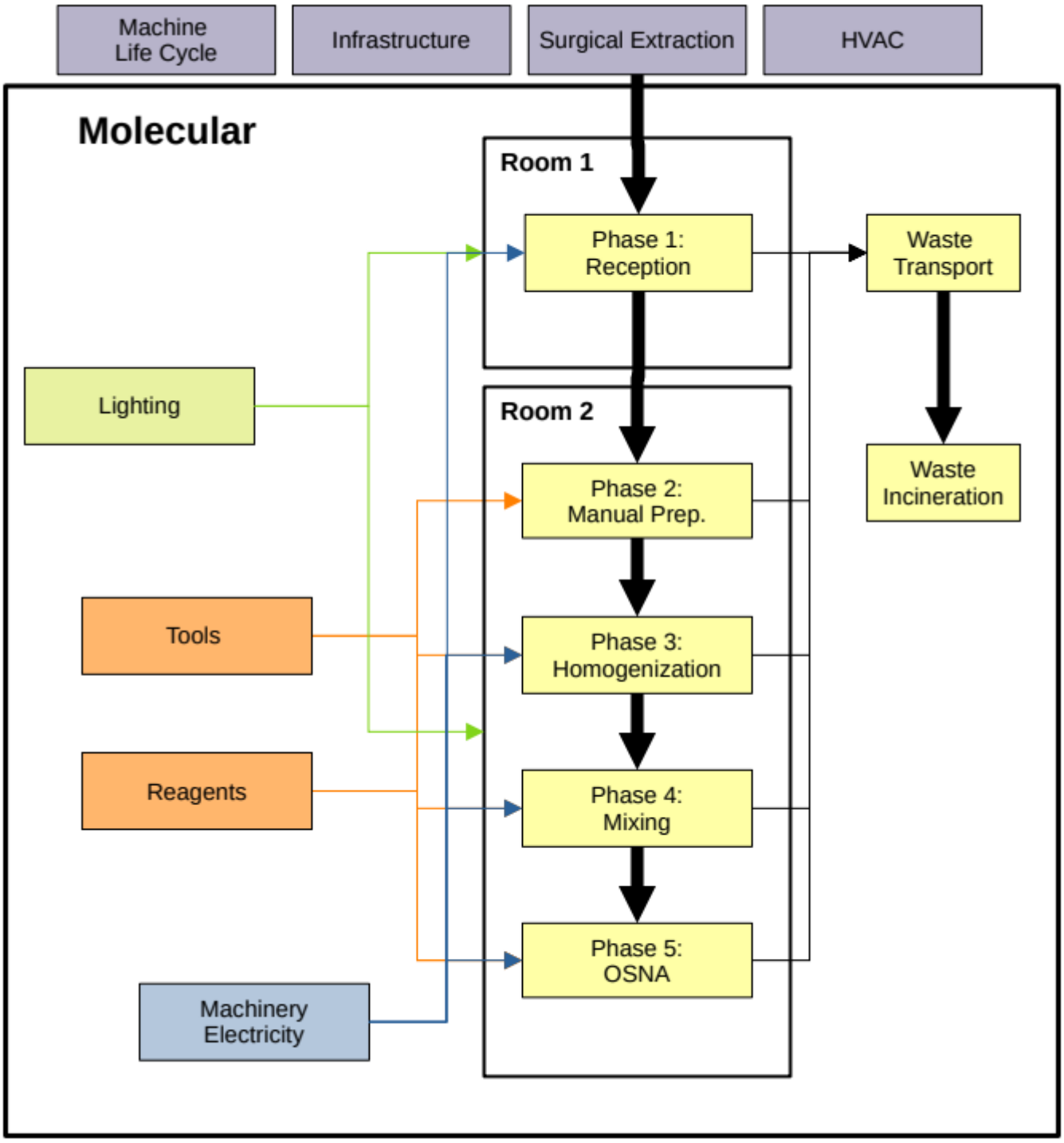

Figure 6: System boundaries – The black box represents the system boundaries, the system is also divided by laboratory room.

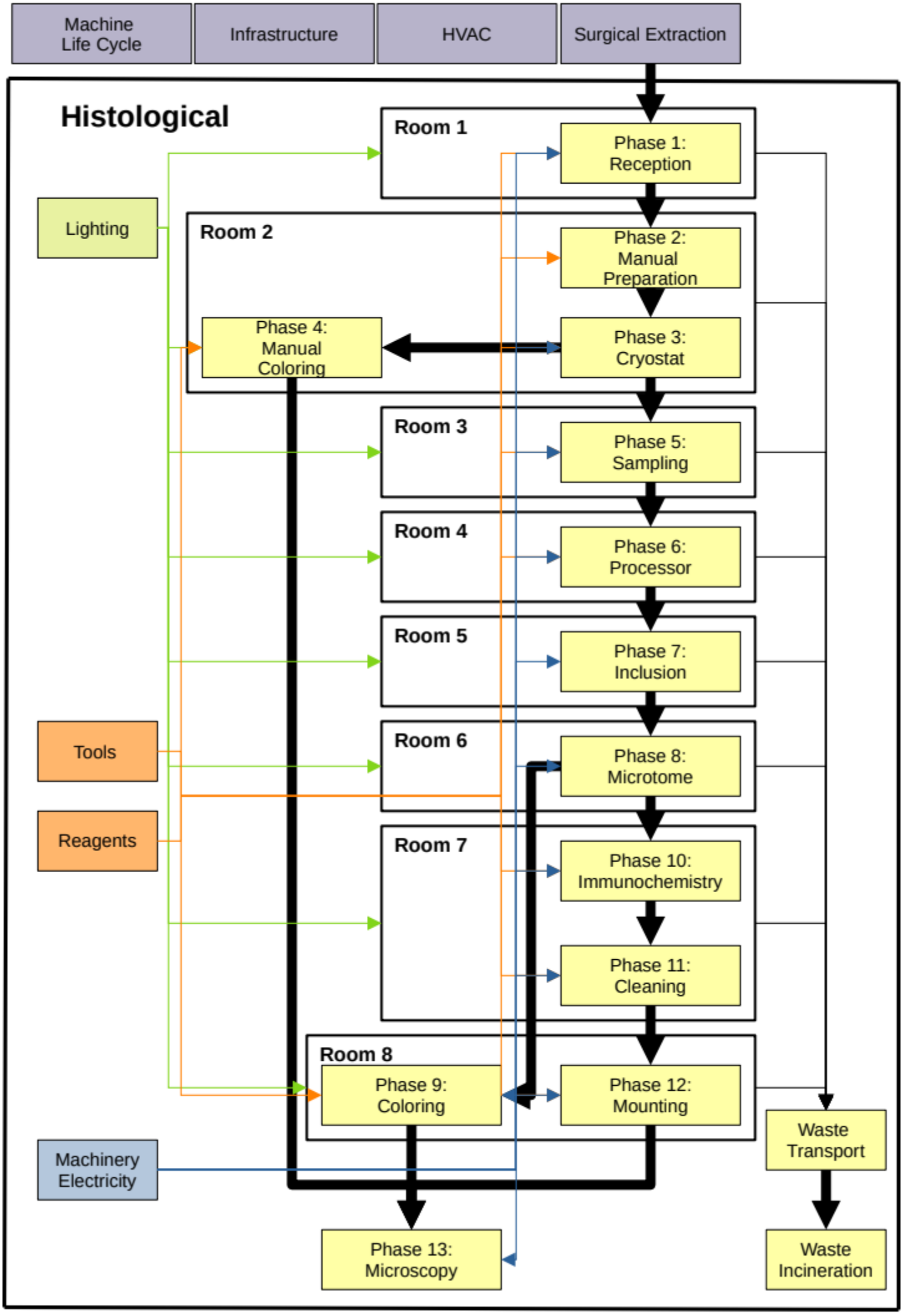

Figure 7 : System boundaries – The black box represent the system boundaries, the system is also divided by laboratory room.

5.2.3 Allocation

In the context of LCA, allocation refers to the process of distributing environmental impacts among multiple products or processes that share a common input or output. This concept is particularly important in scenarios where a single process or system produces more than one product, known as a multi-output system, or when multiple processes share resources[111].

The need for allocation arises because many processes yield multiple outputs, necessitating a way to attribute environmental impacts among the various products. This ensures that the assessment accurately reflects the environmental burden associated with each output.

Several methods can be employed for allocation in LCA:

1. **Physical Allocation** involves distributing impacts based on physical measures, such as mass, volume, or energy content. For instance, in a process that produces two products, the environmental impacts might be allocated according to the mass of each product.
2. **Economic Allocation** assigns impacts based on the economic value of each product, allocating a greater share of the environmental burden to higher-value products.
3. **System Expansion (Avoided Burden)** is an alternative approach that expands the system boundaries to include the benefits of producing secondary products, attributing avoided impacts to the main product instead of allocating them.

In this analysis, the **physical allocation paradigm** is employed, which is particularly relevant when considering that the functional unit is physically divided and subjected to various procedures that collectively contribute to achieving the overall objective. This approach allows for a more precise attribution of environmental burdens to each fraction of the functional unit.

Moreover, applying the physical allocation paradigm allows for a more nuanced understanding of the overall environmental performance of the system. By accounting for the specific procedures each divided portion undergoes, we can identify the weak-points of the system.

6 Chapter : LCI

6.1 Data Types

6.1.1 Foreground

Foreground data refers to the detailed information directly related to the processes and activities under study. Foreground data includes operational details such as energy consumption, material usage, emissions, and waste generation from the processes being analyzed. It also covers process inputs and outputs, including raw materials, energy, water, emissions, waste, and final products.

Foreground data plays a critical role in LCA because of its direct impact on the system being assessed, making accuracy and relevance essential.

6.1.1.1 Sources

The foreground data was collected in accordance with the source prioritization outlined in Figure 8, which emphasizes the use of the most reliable and accurate sources. This hierarchy of data sources was established to ensure that the information included in the assessment reflects the highest level of precision and relevance. Priority was given to primary data obtained directly from the laboratory processes, equipment usage, and operational activities, as this data provides the most detailed and site-specific insights.

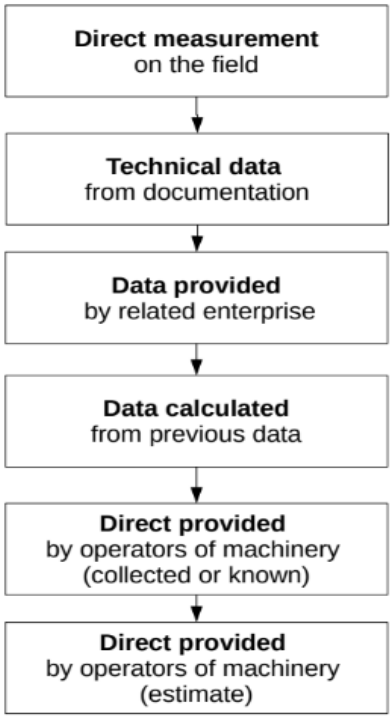

Figure 8: Data Collection Priority - The diagram illustrates from top to bottom the priority of sources for data collection.

6.1.1.2 Limitations

The two main limitation in collecting foreground data arises from the manual nature of the procedures performed by technicians and from the shared nature of the laboratories.

Each technician follows individualized methods for organizing and managing the work environment. These personal variations lead to inconsistencies in how tasks are executed, with procedures not always being carried out in the same manner or with the same level of efficiency. Technicians may have different approaches to using tools, handling materials, or navigating their workspace, which results in fluctuations in performance and resource consumption. Furthermore, day-to-day constraints, such as time pressures or material shortages, can significantly impact operational efficiency.

Compounding these issues is the fact that multiple other procedures are often performed simultaneously in the same laboratory. This overlap in workflows, tool usage, and waste generation introduces an additional layer of complexity.

This combination of day-to-day variability, and overlapping workflows introduces a substantial source of uncertainty into the foreground data. As a result, accurately modeling the system's efficiency and environmental impacts becomes challenging, with this inherent variability significantly influencing the overall uncertainty of the LCA models.

6.1.2 Background

Background data refers to essential information that complements the foreground data, providing a broader context for the assessment. While foreground data focuses on specific measurements and processes directly related to the product or service under study, background data encompasses the upstream and downstream activities associated with the entire life-cycle. This includes details on raw material extraction, energy production, transportation, waste management, and infrastructure related to the production process.

6.1.2.1 Databases

Background data were collected from various external sources, such as publicly available databases like ecoinvent, government and industry reports, and peer-reviewed literature. These sources offer extensive information on LCI data for a wide range of materials, processes, and energy sources.

6.1.2.2 Processes selection

When utilizing data from environmental impact databases, various approaches can be taken based on the context and objectives of the LCA.

One of the critical aspects to consider is the choice of allocation model. Different allocation methods can be applied depending on the specific circumstances and goals of the assessment. In our analysis, we employed a **"cut-off"** model, which aligns with the allocation methodology selected for the remainder of the study. The cut-off model operates under the principle that all environmental burdens associated with the production of a material are allocated entirely to the first user.

When the product reaches its end-of-life and is recycled or reused, the recycled material carries no environmental burden from its prior life cycle. The next user receives it as a burden-free input. This approach simplifies the analysis by focusing on the immediate impacts of the main product, allowing for a clearer understanding of its environmental footprint[115].

In addition to the allocation model, we also meticulously modeled all processes with respect to the appropriate transportation methods. However, in instances where specific transportation details were unavailable, we relied on the **"market"** approach as reported by the relevant database. This means that the assessment incorporates a mean estimation of the environmental burdens associated with the transportation and distribution of the product directly into the process itself. By using this method, we account for the environmental impacts of transportation in a standardized manner, ensuring that our analysis remains robust and comprehensive[116].

6.1.2.3 Limitation

The two primary limitations of utilizing data derived from databases can be collectively attributed to the generalization of the information provided.

Firstly, the data contained in these databases is often derived from mean values, which, by their very nature, may not accurately represent the specific circumstances of the case being studied. While this generalized data can provide useful insights, it may overlook unique characteristics or variations inherent to particular processes or products. To mitigate this issue, one approach is to use data that is geolocalized to the same region as the studied case. This can enhance the relevance of the data by ensuring it reflects regional practices, standards, and environmental conditions. However, even geolocalized data still lacks the reliability and precision of directly measured data, which captures the specific dynamics of a given situation.

Secondly, the generalized nature of database data can obscure the specificity that is often present in real-world supply chains, particularly for products related to healthcare. Healthcare-related products are typically manufactured by a limited number of highly specialized enterprises. These companies often employ unique processes, technologies, and materials tailored to their specific products and markets. As a result, generalized data may not accurately reflect the environmental impacts or resource use associated with these specialized products[117] [118].

6.2 Methods – LCI

The primary data for this study were obtained directly from the clinical pathology laboratory at the IRCCS - Azienda Ospedaliero-Universitaria of Bologna. The data analyzed were collected over a period of one year and reflect the state of operation of the hospital in the 2023.

The quantification of item-based data was performed through direct measurement of each physical item used in the laboratory processes. Weights were determined using a manual precision scale with an accuracy of 0.1 grams, dimensional measurements (length, width, height) were conducted using

a manual ruler with a precision of 1 millimeter, each reported data is an average of three measurement

Electricity consumption was estimated using equipment-specific power ratings provided by the manufacturers. Each machine's energy demand was calculated based on its rated power and average operational time, tailored to its actual usage within the specific diagnostic procedures.

Additionally, the evaluation of the environmental systems operating within the laboratories and waste disposal workflow was informed by architectural and technical documentation from IRCCS planimetry reports, as well as direct data from the hospital's technical and administrative management offices.

The background data were obtained from ecoinvent 3.8.

6.3 Results – LCI

6.3.1 Process layout

The pathology laboratory is organized into specialized rooms, each designated for a specific type of analysis. This layout allows each room to focus on a distinct stage or process in the analysis workflow. As illustrated in Figure 9 for the molecular methodology and Figure 10 for the histological, the sample follows a structured path through the building, moving from one room to the next. In each room, designated procedures are carried out according to the laboratory's protocols. After completing each step, a technician manually transports the sample to the following room for the subsequent phase of analysis.

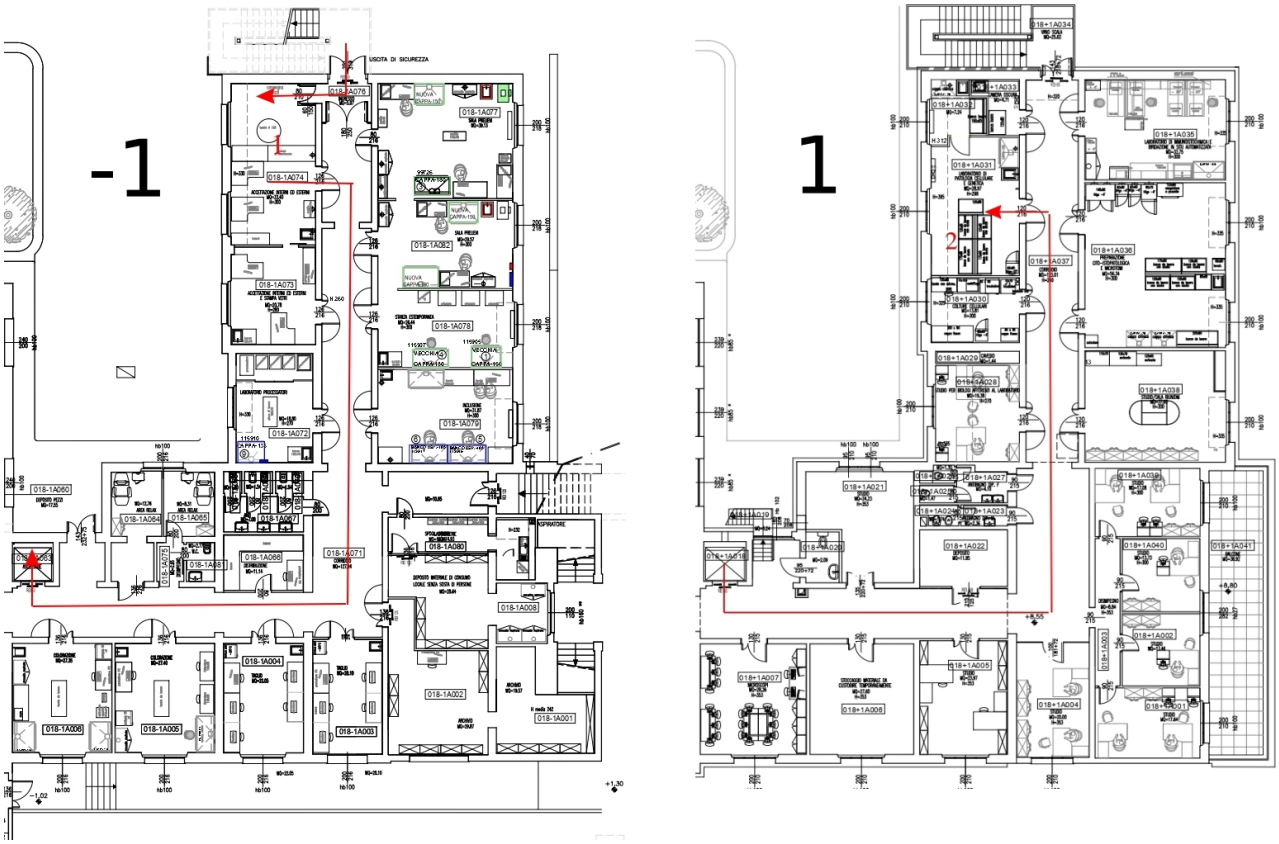

Figure 9: Molecular Spatial Workflow ***-*** *The graph illustrates the movement of the DU in the laboratories spaces and where each procedure is performed:* ***Room 1*** *– Reception,* ***Room 2*** *– Manual cleaning, sample preparation and Molecular methodology analysis.*

Figure 10: Histological Spatial Workflow ***-*** *The graph illustrates the movement of the DU in the laboratories spaces and where each procedure is performed:* ***Room 1*** *– Reception,* ***Room 2*** *– Intraoperative Diagnostics include: Manual cleaning, cryostatic cutting, and H&E staining,* ***Room 3*** *– Sampling,* ***Room 4*** *– Processor: embedding in paraffin,* ***Room 5*** *– Inclusion,* ***Room 6*** *– Microtome cutting,* ***Room 7*** *– Automatic H&E staining and microscopy slide preparation,* ***Room 8*** *– Immunoistochemical analysis and cleaning,* ***Room 9*** *– Automatic microscopy slide preparation.*

6.3.2 Inventory

To ensure a reliable and reproducible inventory analysis, we first conducted an investigation of all inputs and outputs involved in the diagnostic workflow(Figure 11 12 13). This was done through a detailed item-based manual cataloging process performed directly in the laboratory during the procedures, which allowed us to track each component. By organizing the data in this structured manner, we ensured full transparency throughout the analysis, making it easy to trace, understand, and replicate. This approach also enables the findings to be compared more effectively with similar workflows, fostering consistency and facilitating benchmarking against other systems or settings.

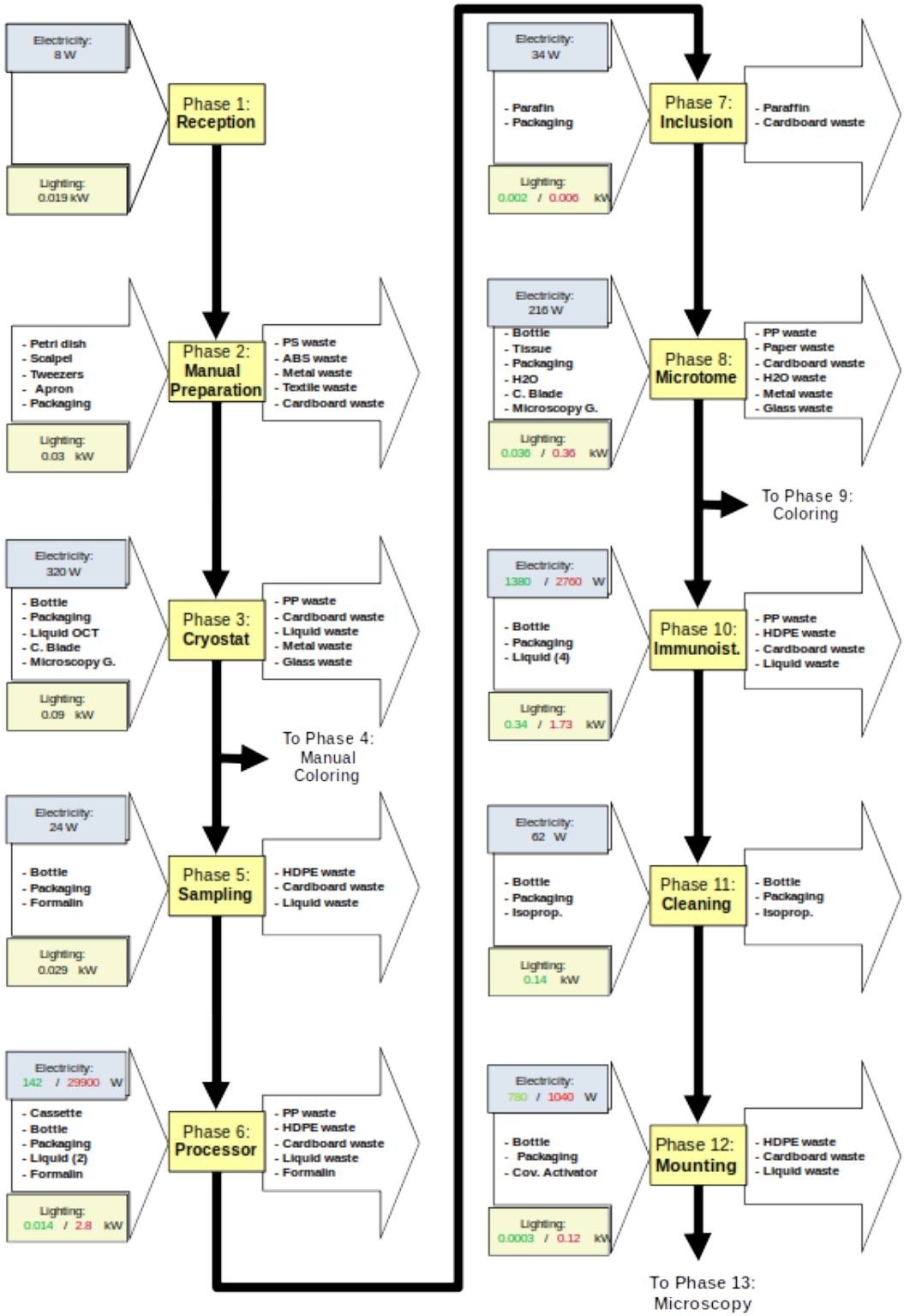

Figure 11: Histological LCI pipeline P.1 - The graph shows an overview of the production pipeline and LCI

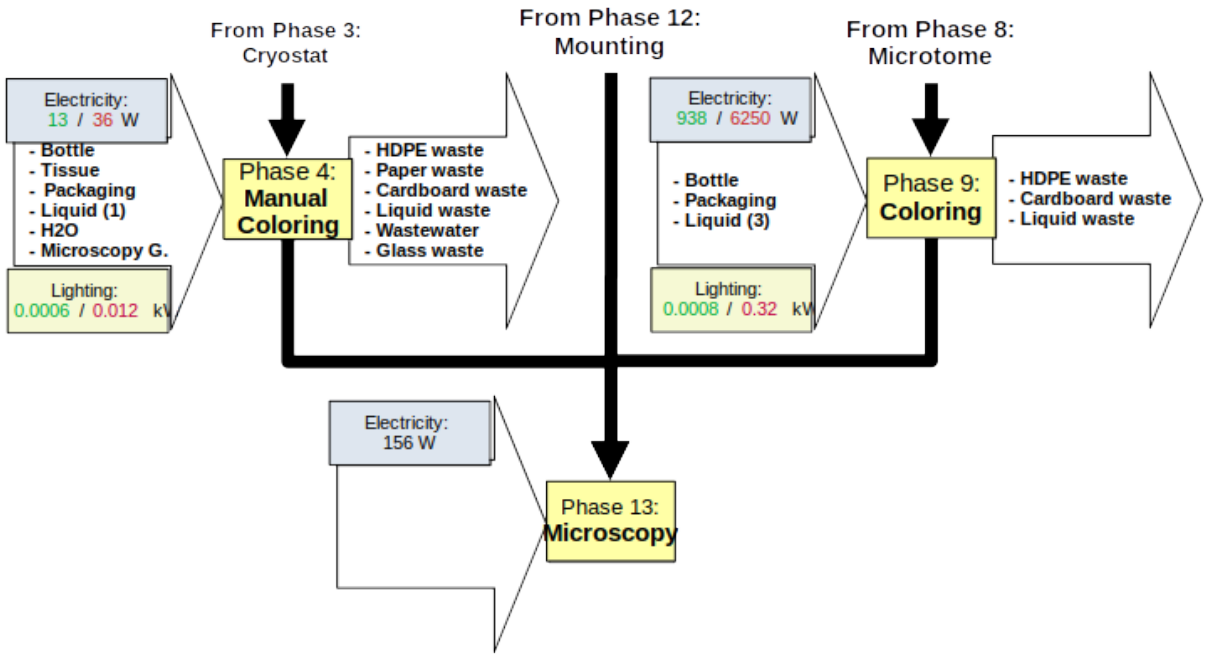

Figure 12 : Histological LCI pipeline P.2 - The graph shows an overview of the production pipeline and LCI

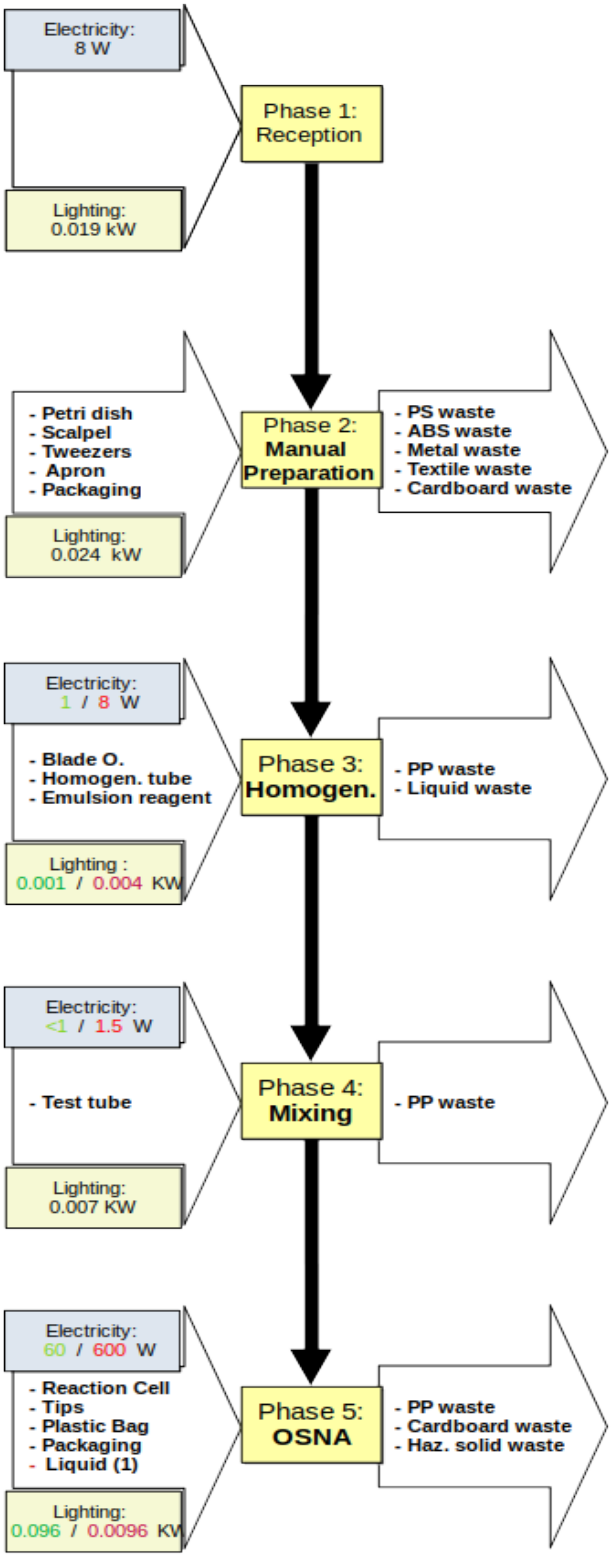

Figure 13: Molecular LCI pipeline - The graph shows an overview of the production pipeline and LCI

In tables 1, 2, 3 a **detailed quantitative inventory** evaluation can bee seen for each of the procedures performed in the studied SNLB methodologies.

Molecular				
Phase 1: Reception				
Machine Electricity	8 Watt			0.019
Phase 2: Manual Prep.				
-Petri dish (PS) -Scalpel and tweezers (ABS+metal) -Apron -Packaging	22g 1 18g + 1g 232g 3g +4g (ABS)	-PS waste -ABS waste -Metal waste -Textile waste -Cardboard waste	22g 22g 1g 232g 3g	0.024
Phase 3: Homogenization				
Machine Electricity -Blade O.(PP) -Lynoprep tube -Lynorag	1 / 8 Wat 10g 6g 4g	-PP waste -Liquid waste	16g 4g	0.0012 / 0.0048
Phase 4: Mixing				
Machine Electricity -Eppendorf (PP)	<1 / 1.5 Watt 1g	-PP waste	1g	0.0072
Phase 5: OSNA				
Machine Electricity -Reaction Cell -Tips -Plastic Bag -Packaging -Liquid (1)	60 / 600 Watt 4.4g / 22g 8.1g / 42g 0.5g / 5g 6.2g 54 / 245 µl	-PP waste -Cardboard waste -Haz. solid waste	13g / 70g 6.2g 54 / 245 µl	0.0096 / 0.096

Table 1: Molecular LCI – Detailed LCI of the molecular methodology

Input		Output		Lighting (kWh)
Histological				
Phase 1: Reception				
Machine Electricity	8 Watt			0.0192
Phase 2: Manual Prep.				
-Petri dish (PS)	22g	-PS waste	22g	0.03
-Scalpel and tweezers	1	-ABS waste	22g	
(ABS+metal)	18g + 1g	-Metal waste	1g	
-Apron	232g	-Textile waste	232g	
-Packaging	3g +4g (ABS)	-Cardboard waste	3g	
Phase 3: Cryostat				
Machine Electricity	320 Watt			0.09
-Bottle (PP)	2g	-PP waste	2g	
-Packaging	17g	-Cardboard waste	17g	
-Liquid OCT	14ml	-Liquid waste	14ml	
-C. Blade	84g	-Metal waste	84g	
-Microscopy G.	35g	-Glass waste	35g	
Phase 4: Manual Coloring				
Machine Electricity	13 / 36 Watt			0.0006 / 0.012
-Bottle (HDPE)	22g	-HDPE waste	22g	
-Tissue	2g	-Paper waste	2g	
-Packaging	25g	-Cardboard waste	25g	
-Liquid (1)	200ml	-Liquid waste	200ml	
-H2O	1200 ml	-Wastewater	1200ml	
-Microscopy G.	75g	-Glass waste	75g	
Phase 5: Sampling				
Machine Electricity	24 Watt			0.029
-Bottle (HDPE)	4.6g	-HDPE waste	4.6g	
-Packaging	4.6g	-Cardboard waste	4.6g	
-Formaline	100ml	-Liquid waste	100ml	
Phase 6: Processor				
Machine Electricity	142 / 29900 Watt			0.014 / 2.8
-Cassette (PP)	2g	-PP waste	2g	
-Bottle (HDPE)	14g	-HDPE waste	14g	
-Packaging	9g	-Cardboard waste	9g	
-Liquid (2)	163ml	-Liquid waste	163ml	
-Formalin	3g	-Formalin	3g	
Phase 7: Inclusion				
Machine Electricity	34 Watt			0.002 / 0.006
-Parafin	5g	-Parafin	5g	
-Packaging	<1g	-Cardboard waste	<1g	

Table 2: Histological LCI 1 – Detailed LCI of the histological methodology

Input		Output		Lighting (kWh)
Phase 8: Microtome				
Machine Electricity	216 Watt			0.036 / 0.36
-Bottle (PP)	13g	-PP waste	13g	
-Tissue	1g	-Paper waste	1g	
-Packaging	24g	-Cardboard waste	24g	
-H2O	100ml	-H2O waste	100ml	
-C. Blade	84g	-Metal waste	84g	
-Microscopy G.	300g	-Glass waste	300g	
Phase 9: Coloring				
Machine Electricity	938 / 6250 Watt			0.0008 / 0.324
-Bottle (HDPE)	28g	-HDPE waste	28g	
-Packaging	7g	-Cardboard waste	7g	
-Liquid (3)	300ml	-Liquid waste	300ml	
Phase 10: Immunoisto.				
Machine Electricity	1380 / 2760 Watt			0.346 / 1.73
-Bottle (PP)	30g	-PP waste	30g	
-Bottle (HDPE)	220g	-HDPE waste	220g	
-Packaging	73g	-Cardboard waste	73g	
-Liquid (4)	2500ml	-Liquid waste	2500ml	
Phase 11: Cleaning				
Machine Electricity	62 Watt			0.144
-Bottle (HDPE)	2g	-HDPE waste	2g	
-Packaging	1g	-Cardboard waste	1g	
-Liquid Isopropanol	19.5ml	-Liquid waste	19.5ml	
Phase 12: Mounting				
Machine Electricity	780 / 1040 Watt			0.0003 / 0.12
-Bottle (HDPE)	1g	-HDPE waste	1g	
-Packaging	<1g	-Cardboard waste	<1g	
-Liquid Cov. Activ.	1.5ml	-Liquid waste	1.5ml	
Phase 13: Microscopy				
Machine Electricity	156 Watt			

Table 3: Histological LCI 2 – Detailed LCI of the histological methodology

6.3.3 End of Life

Clinical pathology laboratory waste falls into two main categories for disposal. The first category includes external packaging materials that have not come into contact with biological samples or reagents, the second category include waste that has come into contact with biologically hazardous materials. Within the bio-hazardous waste category, there is further subdivision into solid and liquid waste, each with specific disposal requirements. Solid bio-hazardous waste, such as tissue or contaminated equipment, can serve as fuel during incineration. In contrast, liquid bio-hazardous waste requires a specialized setup for incineration and additional fuel to ensure safe combustion. Due to these differences in handling, each of the three type of waste is often processed in separate waste disposal facilities designed to meet each waste type's specific requirements. This division implies different methodologies for waste transportation and disposal.

6.3.3.1 Transport

The non hazardous category of waste is treated has standard municipal solid waste and therefore the transport of it has been modeled as a standard market process.

However, when it comes to hazardous waste transportation, the situation is more stable. The IRCCS works with a fixed waste disposal enterprise that handles all hazardous waste generated by the institution. Since this enterprise consistently manages the waste and operates using known processes, we were able to model waste transport based on actual data(Figure 14). The waste transport model reflects the real transportation methods and distances used by this company, providing an accurate representation of the waste disposal activities.

	SOLID WASTE	LIQUID WASTE
Distance Km	76	117
		356
Transport Tonnage	12	8
		28
Engine Type	Diesel E6	Diesel E5
		Diesel VARIABLE

Figure 14: Hazardous Waste Transport - Type of transportation and distance traveled for each of the two type of hazardous waste.

6.3.3.2 Waste disposal

Items classified as non-hazardous can either be recycled or disposed of as regular waste in landfills. These materials are managed as **standard municipal solid waste**, therefore has been modeled as market process for standard municipal solid waste.

On the other hand, solid and liquid hazardous waste is managed through a specialized incineration process. These wastes are sent to two separate incineration plants, each designed to handle specific types of hazardous materials. During incineration, the waste is processed, and any residual ash or debris generated is collected and transported to a landfill designated for final disposal. The incineration process has been modeled as hazardous waste incineration with fly ash extraction, adhering to the operational specifications declared by the managing enterprise.

6.3.4 Lighting

The lighting system at IRCCS utilizes a standardized configuration of light fixtures throughout the entire facility. This approach simplifies maintenance and replacement processes, as any malfunctioning fixture can be quickly and easily serviced or replaced. Each fixture is equipped with four neon tubes, each measuring 50 cm and consuming 0.018 kW/h. Consequently, each fixture operates at a total power consumption of 0.072 kW/h.

Given this uniformity, we assessed lighting consumption on a room-by-room basis. For each room, the total energy consumption for lighting was calculated based on the number of fixtures, their usage duration (which is aligned with the procedural time in each space), and an allocation fraction.

This fraction represents the proportion of SLBN analyses conducted in the room relative to the total number of all procedures carried out there. Only the lighting required during the procedures was computed, this was done to strictly consider only the energy required by the diagnostic workflow excluding other activities. This allocation method ensures that lighting consumption accurately reflects procedural demand in each specific area. Details of the assessment, including room-specific energy usage and allocation fractions, are provided in Figure 15.

Laboratory	Process	Laboratory					Consumption				
		Room 1 (mq)	Room 2 (mq)	TOT (mq)	Operational time (min)	Allocation fraction	N° Lighting points	Single (kw)	N° Lightbulbs	TOT (kW)	KW / FU (worst)	KW / FU (best)
Laboratory 1	1. Reception	33.4	20.78	54.18	2	1	8	0.018	4	0.576	0.0192	0.0192
Laboratory 2	2. Manual Prep				5	1					0.024	0.024
	3. Homogenization	28.97		28.97	1	0.25	4	0.018	4	0.288	0.0048	0.0012
	4. Mixing				1.5	1					0.0072	0.0072
	5. OSNA				20	0.1					0.096	0.0096

Laboratory	Process	Laboratory					Consumption					
		Room 1 (mq)	Room 2 (mq)	TOT (mq)	Operational time (min)	Allocation fraction	N° Lighting points	Single (kw)	N° Lightbulbs	TOT (kW)	KW / FU (worst)	KW / FU (best)
Laboratory 1	1. Reception	33.4	20.8	54.2	2	1	8	0.018	4	0.576	0.0192	0.0192
Laboratory 2	2. Manual Prep				5	1					0.03	0.03
	3. Cryostat	26.4		26.4	15	1	5	0.018	4	0.36	0.09	0.09
	4. Manual coloring				2	0.05					0.012	0.0006
Laboratory 3	5. Sampling	39.1	29.6	68.7	2	1	12	0.018	4	0.864	0.0288	0.0288
Laboratory 4	6. Processor	18.8		18.8	780	0.005	3	0.018	4	0.216	2.808	0.01404
Laboratory 5	7. Inclusion	31.9		31.9	1	0.33	5	0.018	4	0.36	0.006	0.00198
Laboratory 6	8. Microtome	22.1	20.1	42.2	30	0.1	10	0.018	4	0.72	0.36	0.036
Laboratory 7	10. Immunochemistry	27.4	23.1	50.5	120	0.2	12	0.018	4	0.864	1.728	0.3456
	11.Cleaning				10	1					0.144	0.144
Laboratory 8	9. Coloring	27.4		27.4	45	0.0025	6	0.018	4	0.432	0.324	0.00081
	12. Mounting				15	0.0025					0.108	0.00027

Figure 15: Lighting LCI - Detailed evaluation of the Room-by-Room lighting energy use and allocation.

6.4 Input Assumptions

The inputs and outputs in this analysis were modeled based on the known composition and life cycle of the items. However, for the background data, assumptions have to be made. These assumptions are necessary to model processes not directly under our control that are not quantifiable in the present LCA setting.

In the following section, we outline all the assumptions that were made, based on the data available in LCA databases and the similarity of the processes. Where exact data could not be obtained, processes from analogous systems were used to approximate the missing information.

To ensure consistency and relevance, the geolocation of background processes was selected following a prioritized hierarchy. The order of priority was as follows:

1. **[EU] – European Union**: Data specific to EU member states was prioritized to reflect the environmental policies, energy mixes, and manufacturing practices regulated by EU standards.
2. **[RER] – European Region**: If EU-specific data was not available, we used data representative of the broader European region, which includes both EU and non-EU countries. This provides a more general European average.
3. **[GLO] – Global**: In cases where neither EU nor RER data was available, global data was used. This represents worldwide averages and is typically less region-specific but ensures that no critical process data is omitted.

Given that the hospital hosts a diverse range of activities, spanning from patient care to research, the procurement channels for supplies and materials tend to change frequently. This variability makes it challenging to track and investigate the specific transportation methods used for each item delivered to the institution. As a result, we opted to model all system inputs using **market processes**. These market processes represent averaged, generalized supply chain activities commonly used in LCA databases and are suitable when precise transportation data is unavailable. This approach ensures a practical, reliable and standardized estimation of the environmental impacts associated with the procurement of materials.

6.4.1.1 Tools and packaging

In Table 4, the assumptions related to single-use tools and the packaging used for both delivery to the hospital and storage are outlined. Each tool was modeled using an item-based categorization, meaning that each item has been modeled based on the the component it is composed of and the resources needed for the production of one single item.

An exception to this method was made for tools made from **Polypropylene (PP)**. Since these tools consist of a single material and are typically produced through a single, standardized production process, they were modeled based on their weight rather than individual item characteristics. This weight-based approach provided a more streamlined and practical representation for PP tools, as their uniform material composition simplifies the modeling process.

Additionally, the packaging materials used for delivery and storage were also modeled on a weight basis. This decision was made due to the wide variety of packaging shapes and sizes employed, which would be difficult to individually categorize. By modeling packaging by weight, we were able to model al the different types of packaging, regardless of the specific dimensions or configurations.

Card Borad packaging	**1 kg**
Folding boxboard carton [RER] market	1 kg
Carton bord box production with offset printing [GLO] market	0,93 kg
Glass bottle	**0,997 Kg**
Packaging glass [GLO] market	1 kg
Blow moulding [GLO] market	1 kg
HDPE bottle	**0,997 kg**
Polyethylene, high density, granulate [GLO] market	1 kg
Blow moulding [GLO] market	1 kg
PP tools	**0,994 kg**
Polypropylene, granulate [GLO] market	1 kg
injection moulding [GLO] market	1kg
Metal blade	**1 item**
Steel, chromium steel 18/8 [GLO] market	84 g
Metal working, average chromium steel manufacturing [GLO] marke	84 g
Microscopy slides	**1 item**
Flat glass, coated [RER] market	5 g
Tissue	**1 item**
Tissue paper [GLO] market	1 g
Scalpel	**1 item**
Steel, chromium steel 18/8 [GLO] market	1 g
Acrylonitrile butadine styrene [EU]	12g
injection moulding [GLO] market	12g
Metal working, average chromium steel manufacturing [GLO] marke	1g
Tweezers	**1 item**
Acrylonitrile butadine styrene [EU]	8 g
injection moulding [GLO] market	8 g
Medical suit	**1 item**
Textile, nonwoven polypropylene [GLO] market	232 g

Table 4: Input Assumption - Tools and packaging modeling assumption.

6.4.1.2 Reagents

All reagents used in the system were modeled based on product composition specifications provided by the manufacturing companies. Given the limited availability of detailed environmental impact data for medical chemicals, we adopted a proxy approach for the modeling of these substances. Specifically, two representative chemicals were used as proxies: one for organic chemicals and one for inorganic chemicals. These proxies were computed based on the environmental profiles of the 20 most commonly used chemicals worldwide, providing standardized approximation for the chemicals in question.

Additionally, batch-specific chemicals were modeled to account for their specific use within the system.

Certain chemicals, underlined in Figure 16, require medical-grade production standards. As these standards typically involve more stringent processes and higher energy consumption, we applied a **25x multiplier** to their environmental burden, as previously discussed.

The only exception to this approach are the reagents labeled **“OSNA Reagents Pharma,”** which utilizes synthetic RNA in its composition. Due to the complexity of RNA synthesis and its potential to introduce significant variability in environmental impacts, we conducted a complete sensitivity analysis specifically for this reagents. This analysis was performed to better understand the range of potential environmental effects associated with RNA synthesis, ensuring that the model remains robust and that uncertainties are properly accounted for.

Histologic	
Liquid 2 : Process Mix	163 g
Ethanol 99.7% market	108 g
Isopropanol [RER] market	54 g
Liquid 1 : Manual Coloring Mix	200 g
Ethanol 99.7% market	85 g
Toluene, liquid [RER] market	29 g
Water, ultrapure [RER] market	29 g
H&E stain	57 g
Liquid 4 : Immunoisto. Pharma Mix	2500 g
Immunoisto.	2485 g
Immunoisto. Pharma	75 g
Detection Kit	225 g
H&E stain	3 g
Liquid 3 : Coloring Mix	300 g
Coloring	225 g
H&E stain	75 g
Mounting Reagents	3 g

Molecular	
Liquid 1 : OSNA Reagents Pharma	1 kg
Chemical, organic [GLO] market	350 g
Water, ultrapure [RER] market	650 g
OCT	1 kg
Chemical, organic [GLO] market	150 g
Water, ultrapure [RER] market	850 g
Lynorag	1 kg
Chemical, organic [GLO] market	30 g
Water, ultrapure [RER] market	970 g

Secondary reagents Histologic	
Mounting Reagents	1 kg
Chemical, organic [GLO] market	950 g
Water, ultrapure [RER] market	5 g
Immunoisto.	1 kg
Chemical, organic [GLO] market	120 g
Water, ultrapure [RER] market	510 g
White mineral oil	70 g
Immunoisto. Pharma	1 kg
Chemical, organic [GLO] market	10 g
Chemical, inorganic [GLO] market	150 g
Water, ultrapure [RER] market	840 g
H&E stain	1 kg
Chemical, organic [GLO] market	650 g
Chemical, inorganic [GLO] market	10 g
Water, ultrapure [RER] market	340 g
Detection Kit	1 kg
Chemical, organic [GLO] market	670 g
Chemical, inorganic [GLO] market	10 g
Water, ultrapure [RER] market	320 g
Coloring	1 kg
Chemical, organic [GLO] market	590 g
Non-ionic surfactant [GLO] market	70 g
Water, ultrapure [RER] market	340 g

Figure 16: Chemicals Assumption – Modeling assumption for reagents, underscored chemicals refer to chemicals that require pharmaceutical or higher grade chemicals.

7 Chapter : LCIA

7.1 Methods – LCIA

7.1.1 CML baseline

The CML baseline LCA indicators are a fundamental part of a methodology developed by the **Centrum voor Milieukunde Leiden (CML)** at Leiden University. This methodology is designed to assess environmental impacts throughout a product's or process's entire life cycle, from resource extraction to disposal. The **CML-IA (CML Impact Assessment)** method is one of the earliest, most established frameworks for LCA in an European context, offering a structured and standardized approach to evaluating a wide array of environmental indicators.

A critical component of the CML-IA method is the use of characterization factors. These factors quantify the potential environmental impacts associated with specific emissions or resource uses, translating data into measurable impacts (Figure 17). Derived from comprehensive research, these characterization factors undergo periodic updates to incorporate the latest scientific findings, ensuring that the method remains relevant and accurate.

A recent study benchmarked the CML-IA baseline against other prominent LCA methodologies, such as ReCiPe and ILCD. These comparative analyses reveal that while the CML-IA method provides a reliable foundation for impact assessment, methods like ReCiPe offer an expanded perspective by integrating additional endpoints and covering a broader range of impact categories. Despite these differences, the results across methodologies often yield similar conclusions at the midpoint level. This consistency underscores the robustness of the CML-IA framework, making it a practical and dependable choice for various environmental assessments [119].

Moreover, by considering midpoint indicators, we avoiding possible inaccuracies derived from end-point aggregation and obtain a more detailed overview of the diagnostic procedures.

Notably CML-IA do not include normalization giving us an absolute evaluation of the environmental impact. This allow for more transparency and reproducibility in the analysis and avoid relying on ever changing normalization factors.

Impact Category	Units
Abiotic depletion	kg Sb eq
Abiotic depletion (fossil fuels)	MJ
Global warming (GWP100a)	kg CO2 eq
Ozone layer depletion (ODP)	kg CFC-11 eq
Human toxicity	kg 1,4-DB eq
Fresh water aquatic ecotox.	kg 1,4-DB eq
Marine aquatic ecotoxicity	kg 1,4-DB eq
Terrestrial ecotoxicity	kg 1,4-DB eq
Photochemical oxidation	kg C2H4 eq
Acidification	kg SO2 eq
Eutrophication	kg PO4--- eq

Figure 17: CML baseline impact categories - Complete list of the CML baseline impact categories.

CML baseline utilize various metrics to evaluate and manage the potential damage that different substances and practices may cause to ecosystems, human health, and the planet's natural resources. **Abiotic depletion** examines the exhaustion of non-living natural resources, such as metals and minerals, due to their extraction and use. A subset of this, **abiotic depletion (fossil fuels)**, focuses specifically on the depletion of energy resources like coal, oil, and natural gas, measuring the

reduction in available reserves. Both are critical in assessing resource scarcity and long-term availability.

Global warming potential (GWP100a) evaluates the contribution of greenhouse gas emissions to climate change over a 100-year period, using carbon dioxide equivalents (CO_2e) to standardize the warming effects of gases like methane and nitrous oxide. Similarly, **ozone layer depletion (ODP)** assesses the impact of substances such as **chlorofluorocarbons (CFCs)** and halons that degrade the stratospheric ozone layer, which protects the Earth from harmful **ultraviolet (UV)** radiation.

Human toxicity considers the potential health effects of exposure to harmful substances, such as heavy metals or organic pollutants, through air, water, and soil contamination. Ecotoxicity categories complement this by examining the impacts of pollutants on ecosystems. **Freshwater aquatic ecotoxicity** focuses on the toxic effects of chemicals on species in rivers and lakes, while **marine aquatic ecotoxicity** assesses harm to ocean and sea life. **Terrestrial ecotoxicity** evaluates soil contamination and its effects on plants, animals, and microorganisms.

Photochemical oxidation, also known as photochemical smog formation, measures the formation of ground-level ozone caused by reactions between **volatile organic compounds (VOCs)** and **nitrogen oxides (NO_x)** in sunlight. This contributes to air pollution, affecting human health and vegetation. **Acidification**, on the other hand, evaluates the release of acidic substances like **sulfur dioxide (SO_2)** and nitrogen oxides, which lead to acid rain, harming ecosystems and infrastructure.

Finally, **eutrophication** examines the release of nutrients, particularly nitrogen and phosphorus, that stimulate excessive growth of algae in aquatic ecosystems. This process depletes oxygen in water bodies, damaging aquatic life and creating "dead zones."

7.2 Results – LCIA

7.2.1 Histological

	Histological Max	Histological Min	Mean	% Variation
Abiotic depletion kg Sb eq	1.01E-04	7.21E-05	8.64E-05	16.59
Abiotic depletion (fossil fuels) MJ	4.09E+02	1.93E+02	3.01E+02	35.94
Global warming (GWP100a) kg CO2 eq	2.70E+01	1.17E+01	1.93E+01	39.57
Ozone layer depletion (ODP) kg CFC-11 eq	4.98E-07	2.23E-07	3.60E-07	38.15
Human toxicity kg 1,4-DB eq	1.00E+02	7.56E+01	8.78E+01	13.91
Fresh water aquatic ecotox. kg 1,4-DB eq	2.24E+01	1.73E+01	1.99E+01	12.71
Marine aquatic ecotoxicity kg 1,4-DB eq	4.53E+04	3.13E+04	3.83E+04	18.32
Terrestrial ecotoxicity kg 1,4-DB eq	1.34E+00	1.17E+00	1.26E+00	6.71
Photochemical oxidation kg C2H4 eq	5.86E-03	3.05E-03	4.46E-03	31.60
Acidification kg SO2 eq	8.24E-02	3.76E-02	6.00E-02	37.33
Eutrophication kg PO4--- eq	2.60E-02	1.45E-02	2.02E-02	28.46

Table 5: LCIA Histological - The table report the results of the two scenarios for the Histological methodology with mean value and percentage variation among the two scenarios.

The analysis of the two extreme case scenarios (Table 5) revealed a significant and inconsistent variation across the various impact categories for the analysis of one DU. The variation ranged from a 6.7% difference in Ecotoxicity to a much larger 39.57% difference in Global Warming potential. This wide variation highlights the specificity of the parameters most affected in the scenario analysis. The primary factors contributing to these differences were the amount of electricity consumed by each DU and the volume of liquid hazardous waste generated. These elements were particularly influential in driving the discrepancies observed in the environmental impact across the different scenarios.

7.2.1.1 Global warming (GWP100a)

A noticeable and significant difference emerges between the two scenarios in the **Global Warming Potential (GWP100a)** index, with the primary variation being attributed to the "Processor" process (Figure 18). This discrepancy can be explained by the high capacity of the machine involved in both “Processor” and “Coloring ”, which, when combined with the inherently energy-intensive nature of the processing stage, leads to considerable fluctuations in emissions. The energy consumption during this phase is substantial, and the efficiency of the machine directly impacts the overall environmental footprint in terms of carbon dioxide equivalent emissions.

Running the machine at varying load levels can have a profound effect on the overall GWP100a outcome. When the machine operates at full capacity, energy use and carbon emissions are maximized, whereas operating at a lower load can reduce energy consumption and emissions, leading to a lower global warming impact. This highlights the importance of optimizing machine load during production to minimize the environmental impact, particularly in processes that are heavily reliant on high energy inputs.

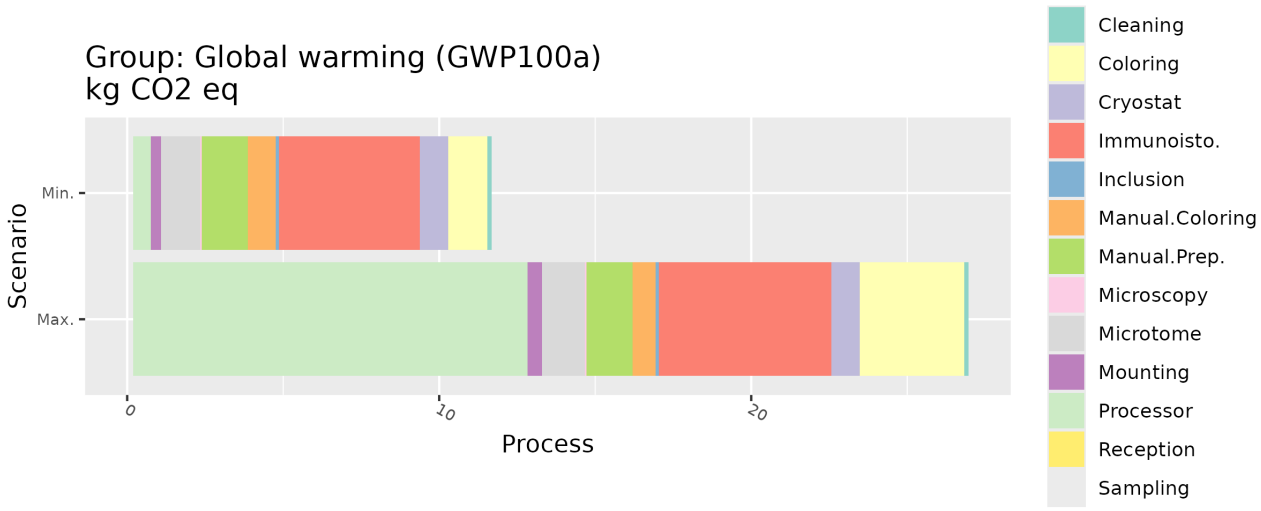

Figure 18: Global Warming - Histological methodology GWP100 results divided by process.

7.2.1.2 Abiotic depletion

When examining Abiotic Depletion in the two scenarios (Figure 19), we observe that the impact is relatively moderate, with values ranging from e-05 to e-04. This is not surprising, as the procedure does not involve the consumption of large quantities of materials, which typically contributes to higher resource depletion. The low-to-moderate range suggests that the overall material consumption for the system is not a major driver of resource depletion.

However, it is noteworthy that the most affected processes between the two scenarios are "Processor" and "Coloring", both of which are the most energy-intensive processes in the system. Despite the moderate abiotic depletion values, these processes stand out due to their high energy consumption, which may indirectly lead to a higher use of non-renewable resources, especially if the energy sources are derived from fossil fuels.

This suggests that, although the materials required for the process are minimal, the energy-intensive nature of certain operations can still result in significant impacts on abiotic depletion.

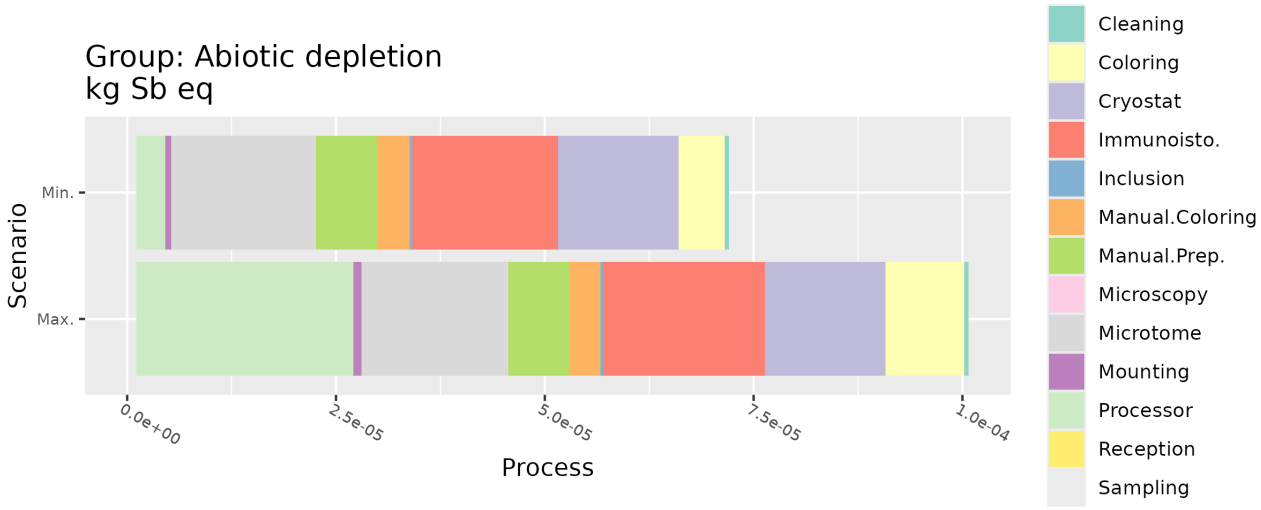

Figure 19: Abiotic Depletion - Histological methodology results for each scenario divided by process.

7.2.1.3 Abiotic depletion (fossil fuels)

We can observe a moderate depletion of fossil fuels (Figure 20), ranging between 200 to 400 MJ, indicating a moderate but not overwhelming reliance on non-renewable energy sources. As anticipated from the previous index, the primary contributors to this depletion are the "Processor" and "Coloring" stages, which are characterized by high energy intensity due to the lenghty and energy intensive processes involved. These stages require considerable amounts of electricity, which explains their notable impact on fossil fuel consumption.

It is important to highlight that the Italian energy mix was used in the modeling, which provides critical insight into the ongoing energy challenges in Italy. Despite advancements in renewable energy, the results underscore the continued substantial dependency of Italian energy production on fossil fuels[120]. This dependence contributes to the depletion of fossil resources, signaling the need for more sustainable energy practices and alternative energy sources within Italy's energy infrastructure to move towards a less invasive and sustainable future.

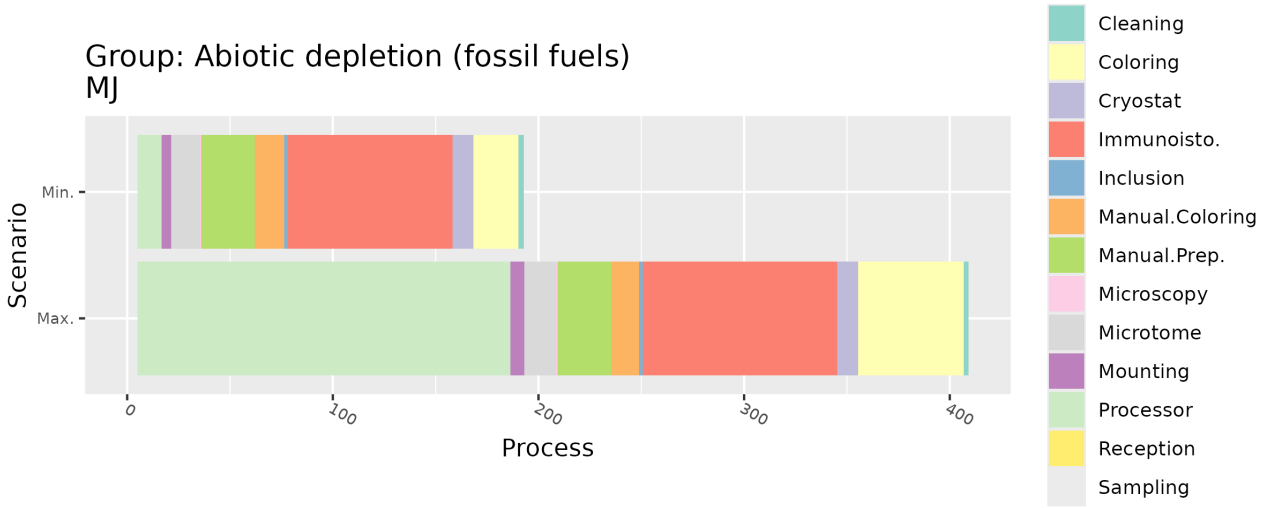

Figure 20: Abiotic Depletion (fossil fuel) - Histological methodology results for each scenario divided by process.

7.2.1.4 Ozone layer depletion

The ozone layer depletion index (Figure 21) reveals an overall low environmental impact, with values ranging between 2e-07 and 5e-07, reflecting the limited contribution of the analyzed system to the emission of ozone laye depleting sudstances. This relatively minor impact can be attributed to the moderate levels of airborne emissions generated by the processes under review.

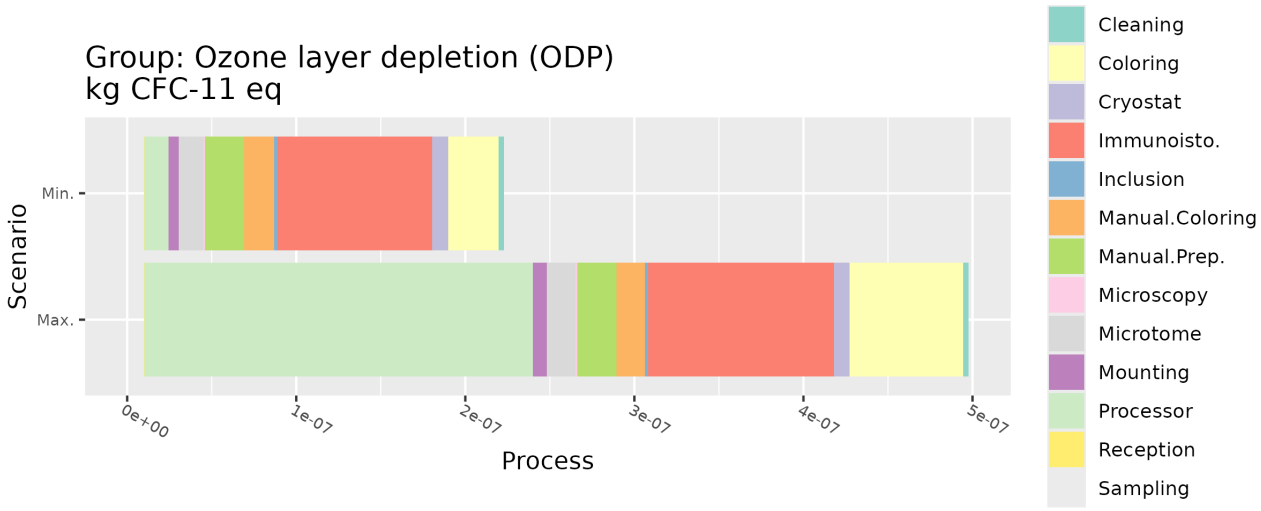

Figure 21: Ozone Layer depletion - Histological methodology results for each scenario divided by process.

7.2.1.5 Human toxicity

Human toxicity (Figure 22) is undoubtedly one of the most significant and intriguing indices in this context, particularly because it shows a moderately high impact when compared to the other environmental categories.

Upon closer examination, it becomes clear that processes such as "Coloring" and "Immunohistochemical" contribute the most to the human toxicity impact. These processes are chemically intensive, relying on substances that can be hazardous to human health if not managed properly.

In addition, the use of large quantities of manufactured glassware in some of the processes further exacerbates the human toxicity impact. The production[121] [122]of glassware often involve the use of toxic materials in their manufacturing process, which can add to the overall chemical burden.

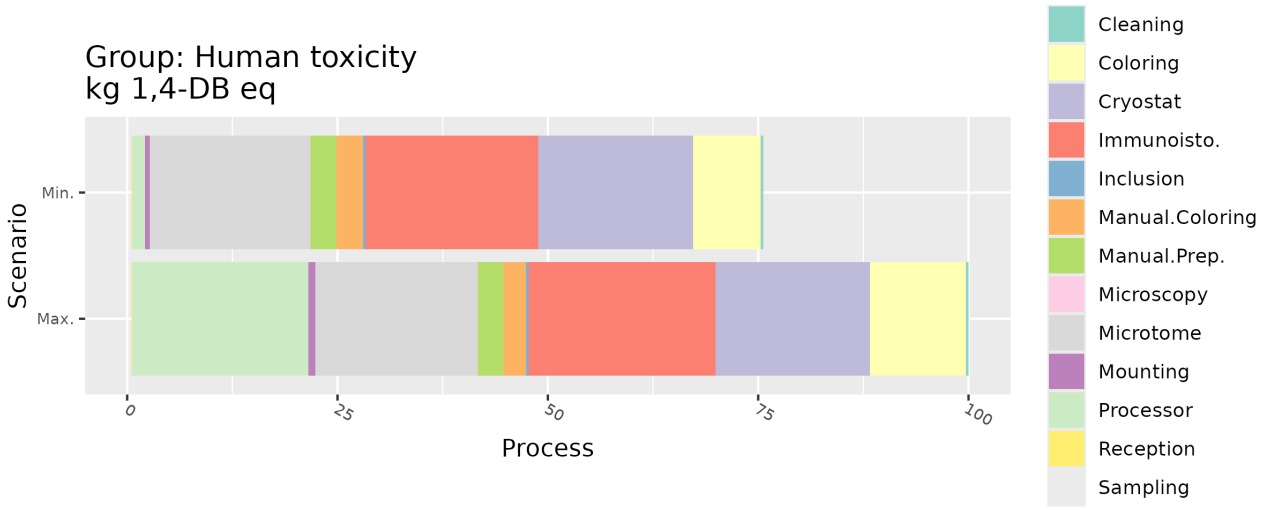

Figure 22: Human Toxicity - Histological methodology results for each scenario divided by process.

7.2.1.6 Fresh water aquatic ecotox.

For this index (Figure 23), we observe that processes involving high chemical usage contribute significantly to freshwater ecotoxicity. The ecotoxicity results fall within the range of 17–20 kg 1,4-DB eq, which suggests a moderate impact on freshwater ecosystems. This range indicates that while the effects are not extreme, they are still substantial enough to pose risks to aquatic life.

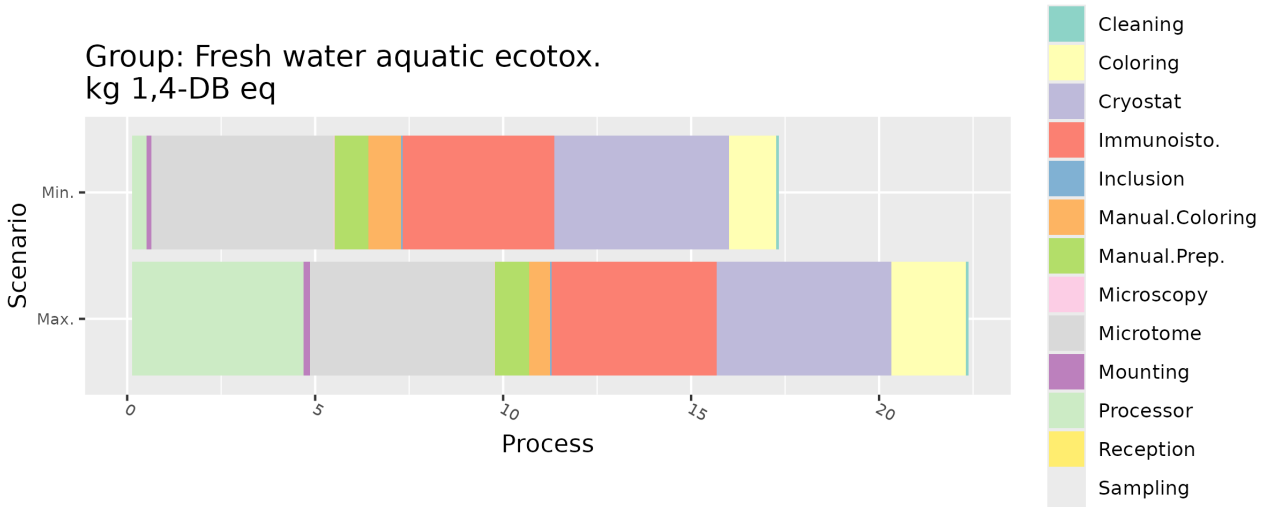

Figure 23: Fresh Water Ecotoxicity - Histological methodology results for each scenario divided by process.

7.2.1.7 Marine aquatic ecotoxicity

The Marine Aquatic Ecotoxicity (Figure 24) index stands out as the most concerning among all impact categories, with significantly higher values compared to the others. This elevated ecotoxicity highlights a critical issue associated with histological methodologies: the high consumption of chemicals and the extensive water required to clean and dilute these substances. Each stage of the histology process, from tissue fixation to staining, involves various chemicals that ultimately lead to large volumes of contaminated wastewater.

This wastewater, containing chemical residues and potentially toxic substances, must be treated and disposed of as biologically hazardous waste, adding to environmental and operational burdens. The challenge of managing this waste is intrinsic to the molecular methodology used in histology, where specific chemical treatments are necessary to achieve accurate staining and analysis of biological tissues. Unfortunately, minimizing this impact is complex, as current histological processes inherently depend on these chemicals and the water required for dilution and rinsing.

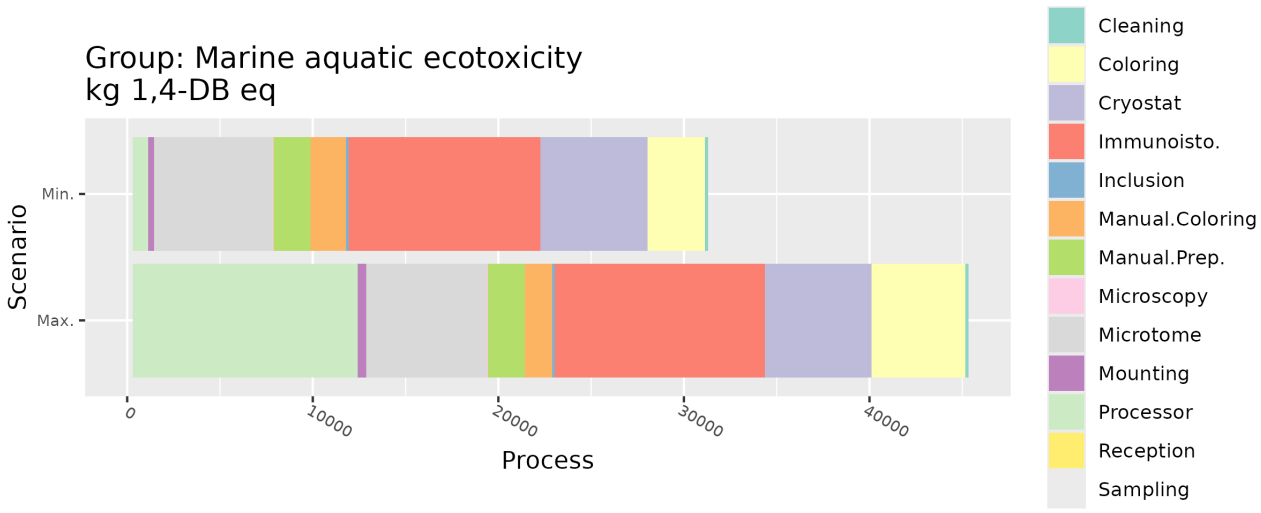

Figure 24: Marine Aquatic Ecotoxicity - Histological methodology results for each scenario divided by process.

7.2.1.8 Terrestrial ecotoxicity

The Terrestrial Ecotoxicity (Figure 25) index shows an overall low impact, suggesting minimal ecological harm to land environments. However, it is interesting to note the primary contributors to this category, specifically the “Microtome” and “Cryostat” processes. These two procedures rely heavily on specialized, single-use cutting tools and a large number of microscopy slides, both of which require extensive mineral extraction and processing.

The production of these consumables involves complex supply chains for extracting and refining minerals, such as silica, metals, and other raw materials, which can have downstream effects on soil quality and biodiversity in mining areas. Even though the terrestrial ecotoxicity impact appears low, the dependence on single-use components and resource-intensive manufacturing processes underscores an indirect but significant environmental footprint.

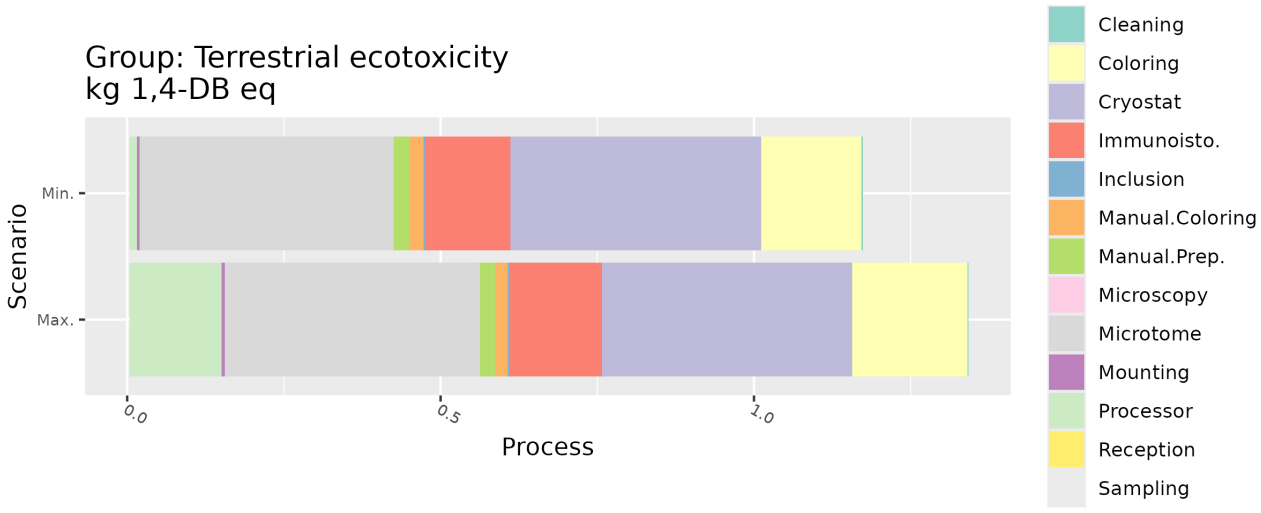

Figure 25: Terrestrial Ecotoxicity - Histological methodology results for each scenario divided by process.

7.2.1.9 Photochemical oxidation

The system shows a low impact in terms of Photochemical Oxidation (Figure 26), which correlates with moderate emission levels. This outcome indicates a relatively small contribution to ground-level ozone formation, a pollutant that can lead to smog and respiratory health issues. Analysis reveals that the most energy-intensive processes are the primary contributors to this index, reaffirming that a significant portion of these emissions stems from electricity generation required to power the system.

The correlation between energy usage and emissions highlights the dependence of the system on electricity, where fossil-fuel-based power sources are likely a major factor.

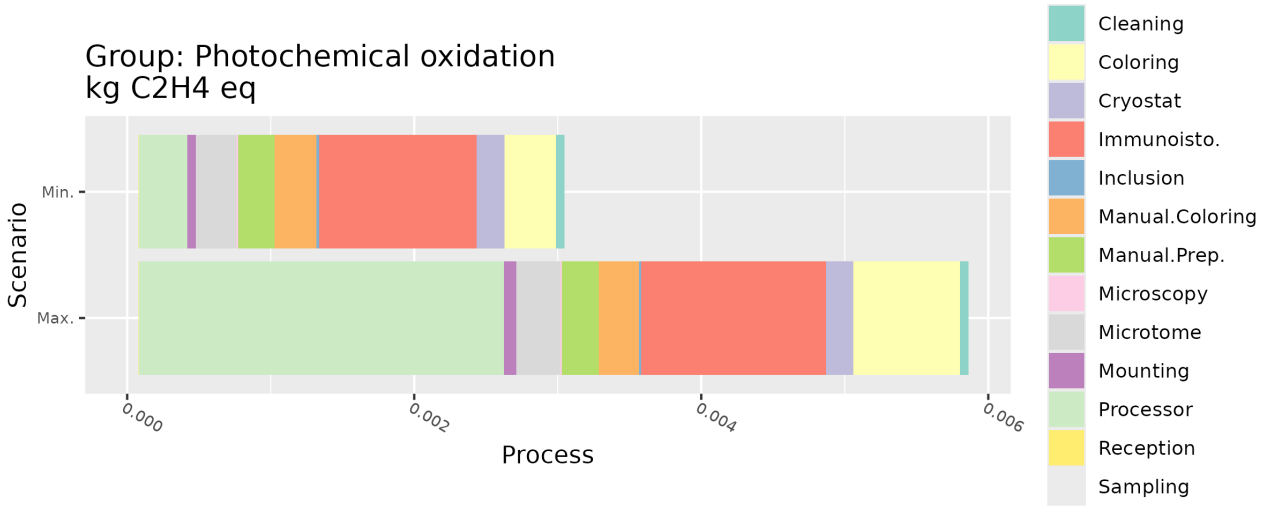

Figure 26: Photochemical Oxidation - Histological methodology results for each scenario divided by process.

7.2.1.10 Acidification

Examining the Acidification (Figure 27) impact factor reveals a pattern similar to that observed in Photochemical Oxidation, with energy-intensive processes as the main contributors. However, the “Processor” process, in particular, has an even greater influence on acidification than it does on photochemical oxidation. This elevated impact highlights a second major issue inherent to the molecular methodology: the use of formalin.

Formalin, a common chemical in the “Processor” stage for tissue preservation, releases compounds that contribute significantly to acidification. Acidifying emissions, such as sulfur and nitrogen oxides, can lead to acid rain, which harms soil, vegetation, and aquatic ecosystems. This reliance on formalin not only affects air quality but also introduces further environmental and health challenges due to its toxicity. Minimizing or finding alternatives to formalin in the molecular methodology could substantially reduce the acidification impact, offering a pathway to make these processes more environmentally sustainable.

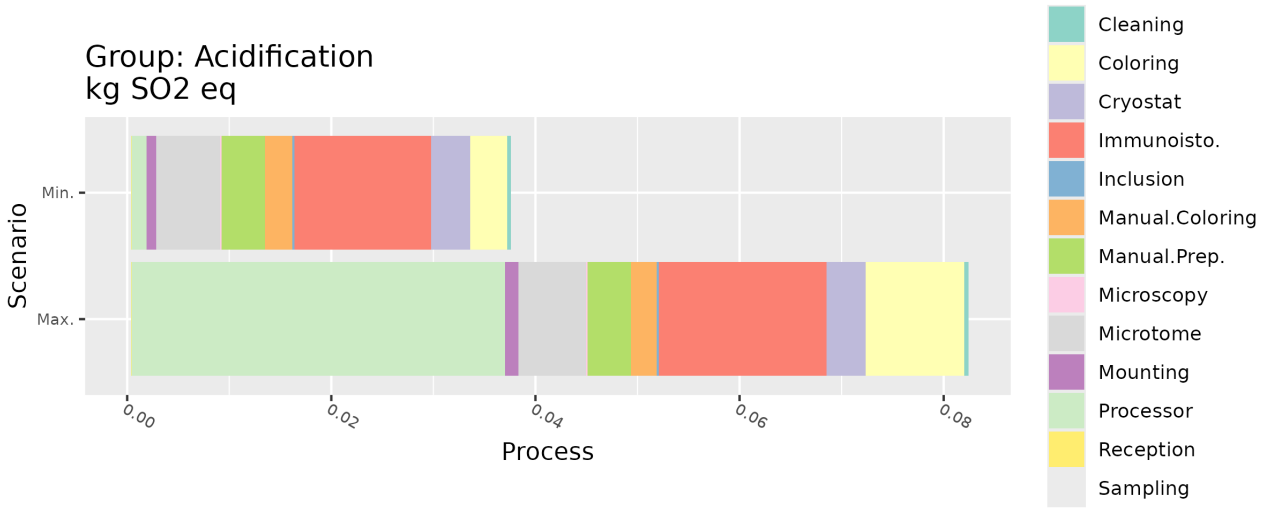

Figure 27: Acidification - Histological methodology results for each scenario divided by process.

7.2.1.11 Eutrophication

The Eutrophication (Figure 28) impact factor once again underscores the environmental challenges already identified, particularly the high energy demands of certain processes. Processes such as “Processor,” “Immunohistochemistry,” and “Coloring” are the largest contributors to eutrophication. This impact arises primarily from nutrient-rich emissions, which can enter aquatic ecosystems and promote excessive algae growth, leading to oxygen depletion and biodiversity loss.

The high energy requirements of these processes contribute indirectly to eutrophication through emissions associated with electricity production, especially if the power sources involve fossil fuels. Additionally, some chemical reagents used in these procedures contain nitrogen or phosphorus compounds that further enhance Eutrophication

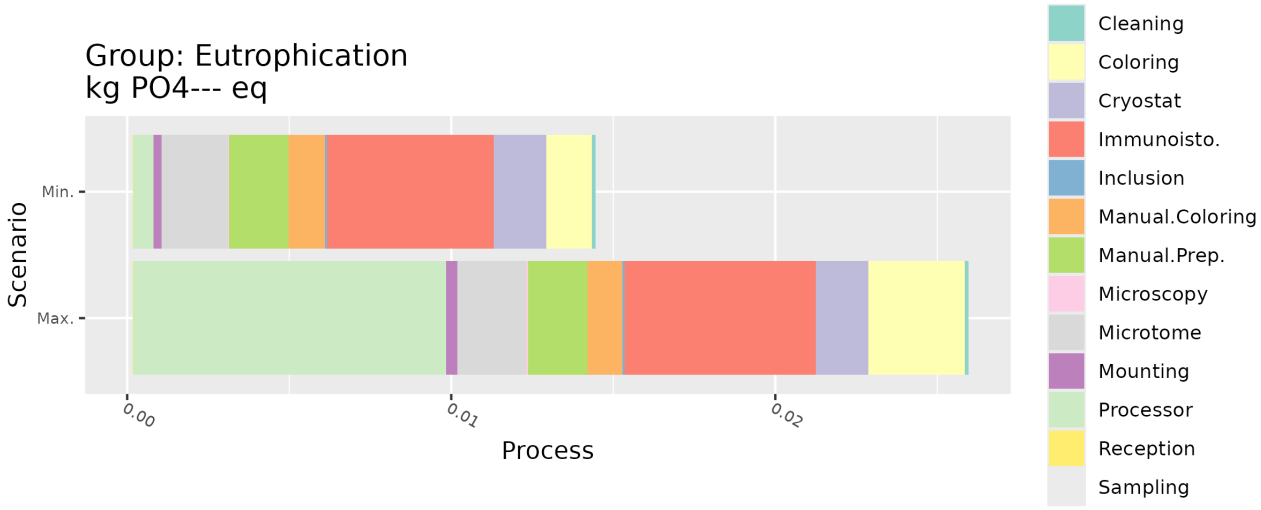

Figure 28: Eutrophication - Histological methodology results for each scenario divided by process.

7.2.2 Molecular

	Molecular Max	Molecular Min	Mean	% Variation
Abiotic depletion kg Sb eq	1.08E-05	8.60E-06	9.69E-06	22.40
Abiotic depletion (fossil fuels) MJ	4.15E+01	3.13E+01	3.64E+01	27.90
Global warming (GWP100a) kg CO2 eq	2.60E+00	1.86E+00	2.23E+00	32.80
Ozone layer depletion (ODP) kg CFC-11 eq	4.05E-08	2.91E-08	3.48E-08	32.60
Human toxicity kg 1,4-DB eq	5.40E+00	3.94E+00	4.67E+00	31.20
Fresh water aquatic ecotox. kg 1,4-DB eq	1.79E+00	1.23E+00	1.51E+00	37.10
Marine aquatic ecotoxicity kg 1,4-DB eq	3.67E+03	2.61E+03	3.14E+03	33.90
Terrestrial ecotoxicity kg 1,4-DB eq	4.20E-02	3.13E-02	3.67E-02	29.00
Photochemical oxidation kg C2H4 eq	4.31E-04	3.16E-04	3.74E-04	30.80
Acidification kg SO2 eq	6.74E-03	5.07E-03	5.90E-03	28.30
Eutrophication kg PO4--- eq	2.77E-03	2.17E-03	2.47E-03	24.20

Table 6: LCIA Molecular - The table report the results of the two scenarios for the Histological methodology with mean value and percentage variation among the two scenarios.

The results for the Molecular methodology (Table 6) reveal a notably stable percentage variation across the evaluated impact indexes, with values ranging from 22.4% to 37.1%. This consistency can be attributed to the simpler and smaller-scale nature of the molecular production system. Unlike more complex methodologies, the Molecular methodology does not involve high-capacity machinery or extensive manual processes, which are prone to variability based on technician practices and operational differences.

In the Molecular methodology, the primary factor influencing impact variation is the number of samples analyzed in a single batch, rather than fluctuations introduced by machine usage or human intervention. This streamlined approach results in less operational variability, making environmental impacts more predictable and stable across different runs.

7.2.2.1 Global warming (GWP100a)

The overall Global Warming Potential impact (Figure 29) is relatively low, indicating that the system does not contribute heavily to climate change. However, upon closer examination, the process with the most significant impact is "Manual Preparation." This process stands out due to the use of single-use tools and garments, which are required for each operation. Moreover we can see that in the maximum impact scenario the “Molecular methodology” process arise to relevance for the same reason. These disposable items, such as gloves, lab coats, test tubes and reaction cells, add to the environmental footprint, as their production, transportation, and disposal all generate greenhouse gas emissions.

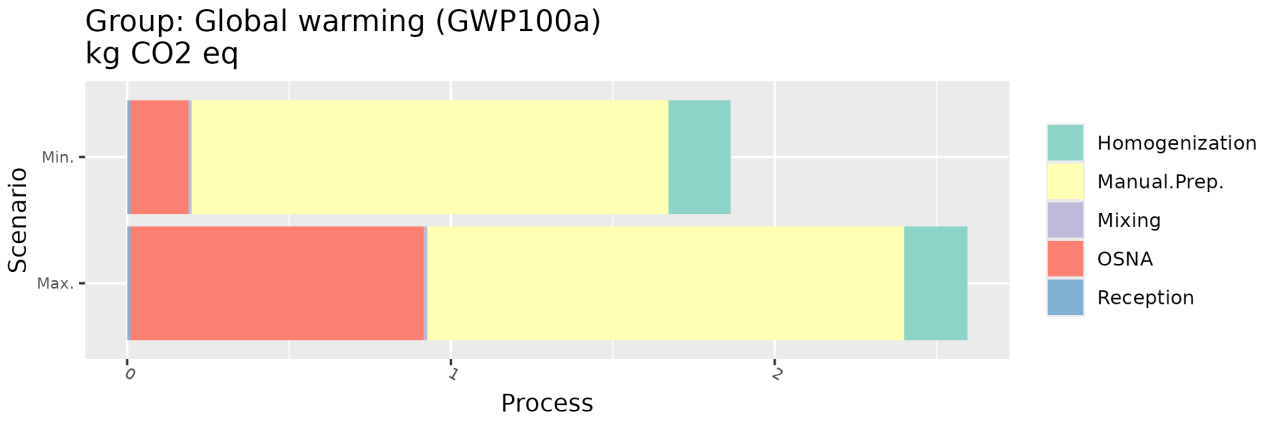

Figure 29: Global Warming - Molecular methodology GWP100 results divided by process.

7.2.2.2 Abiotic depletion

The majority of the Abiotic Depletion impact (Figure 30) is driven by the "Manual Preparation" process. This is primarily due to the extensive use of single-use tools and garments, which require the extraction and processing of raw materials, contributing to the depletion of non-renewable resources. Since the "Manual Preparation" process remains unchanged between the two scenarios, the impact on Abiotic Depletion stays relatively constant. As a result, Abiotic Depletion shows the lowest percentage variation across all the impact categories in the assessment.

This stability indicates that, regardless of other factors or process adjustments in the system, the dependency on single-use materials in "Manual Preparation" consistently contributes to the depletion of non-renewable resources.

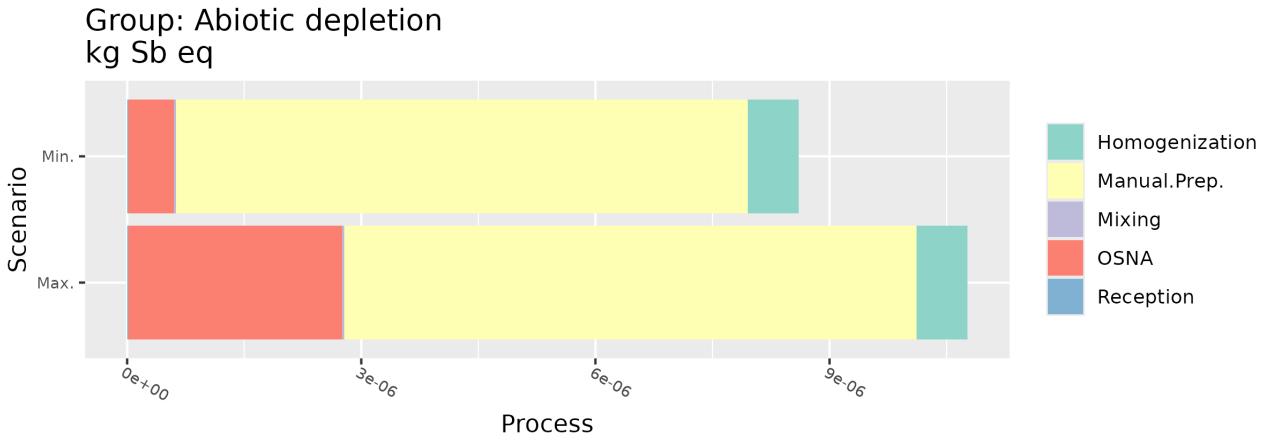

Figure 30: Abiotic Depletion - Molecular methodology results for each scenario divided by process.

7.2.2.3 Abiotic depletion (fossil fuels)

The analysis of fossil fuel depletion (Figure 31) reveals an interesting phenomenon. Although the "Molecular methodology" process is the primary consumer of electricity in the system, the main contributor to fossil fuel depletion is still the "Manual Preparation" process. This may seem counterintuitive at first, given the high electricity demand of the "Molecular methodology" process. However, the significant impact of "Manual Preparation" on fossil fuel depletion is primarily due to the extensive use of plastic materials, which require considerable amounts of fossil fuels for their production.

The manufacturing of plastic items, such as single-use tools and garments, involves the extraction and processing of petroleum and natural gas, both of which are fossil fuels. This process is highly energy-intensive, contributing to the depletion of these resources. In this case, the environmental impact of plastic production in "Manual Preparation" outweighs the electricity consumption in the "Molecular methodology" process, highlighting the substantial fossil fuel usage involved in producing plastic products.

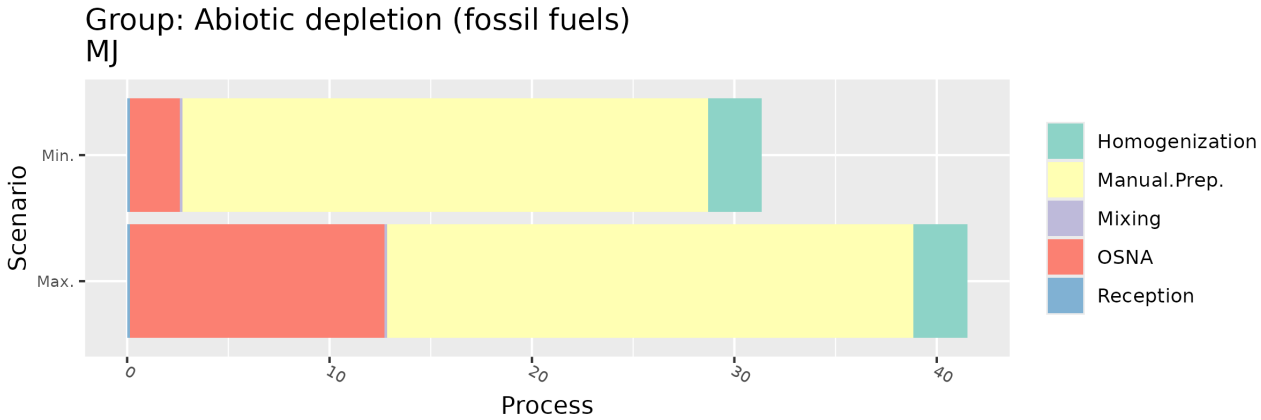

Figure 31: Abiotic Depletion (fossil fuel) - Molecular methodology results for each scenario divided by process.

7.2.2.4 Ozone layer depletion

As observed in Figure 32, the system has a minimal effect on ozone layer depletion, indicating that its contribution to this environmental issue is negligible. This finding confirms that the system itself generates almost no direct emissions that would impact the ozone layer, aside from those accounted for in the background processes.

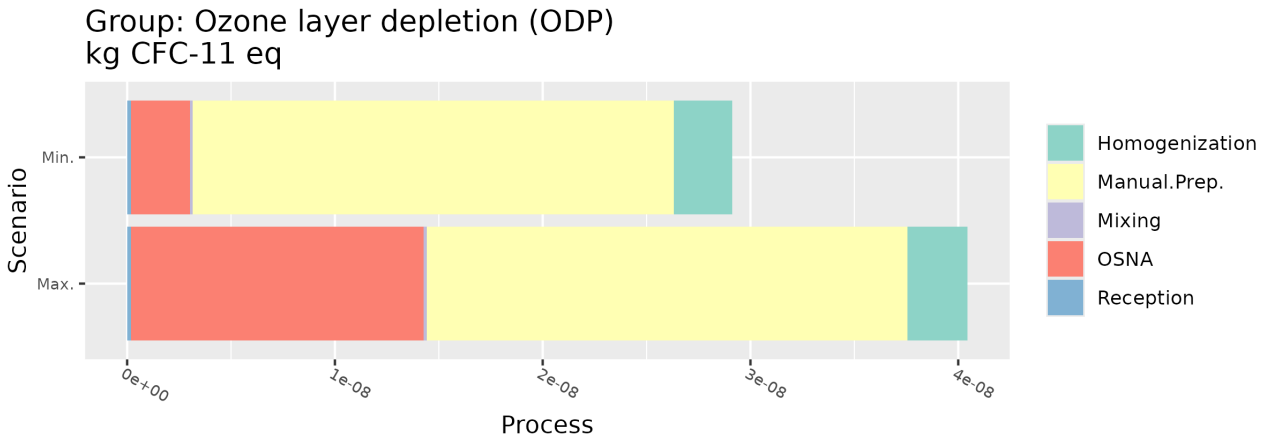

Figure 32: Ozone Layer depletion - Molecular methodology results for each scenario divided by process.

7.2.2.5 Human toxicity

The human toxicity (Figure 33) index is notably higher compared to other environmental impact metrics in this assessment. This increase is partially due to the presence of certain chemicals, which, although used in small quantities, contribute to toxic emissions. However, the primary driver of this heightened toxicity is the incineration of plastic materials. Many of the tools and components assessed are made of single-use plastics, which, when contaminated, require incineration as a disposal method.

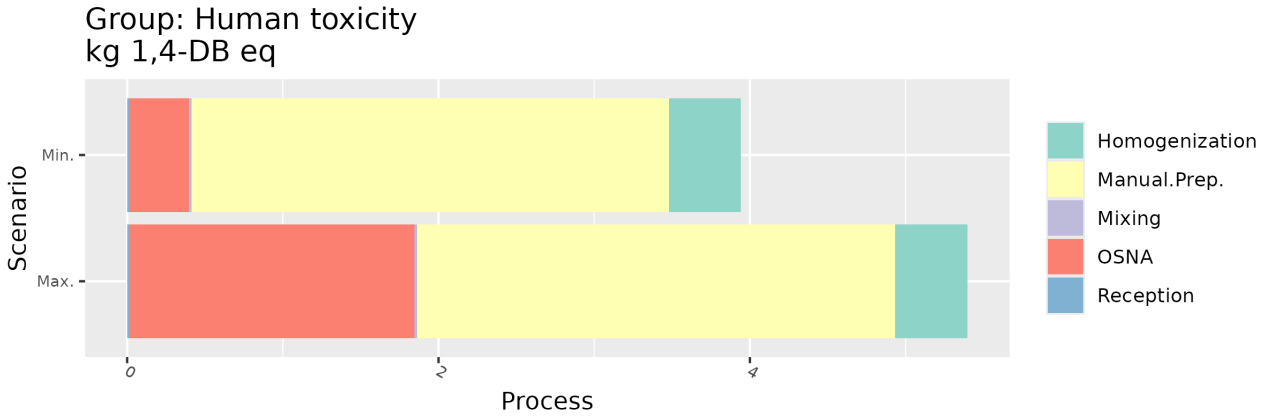

Figure 33: Human Toxicity - Molecular methodology results for each scenario divided by process.

7.2.2.6 Fresh water aquatic ecotoxicity.

Freshwater ecotoxicity (Figure 34) is also impacted by the same parameters that contribute to human toxicity, although its effects are somewhat less pronounced. The presence of specific chemicals, even in small amounts, along with the incineration of plastic materials, plays a significant role in this impact. When plastics incineration residues and associated chemicals enter freshwater systems, either through emissions from incineration or leaching from waste disposal sites, they can introduce toxic substances that harm aquatic life.

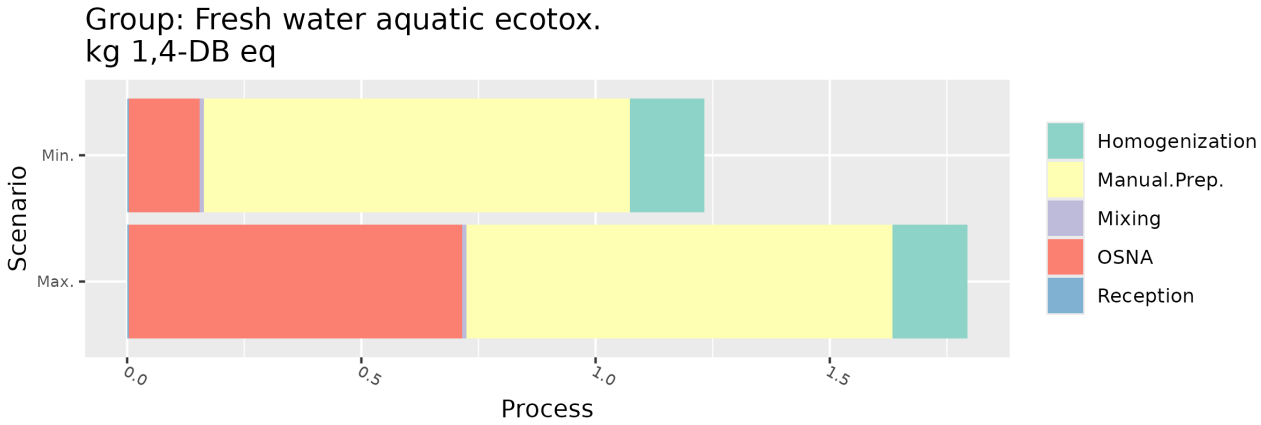

Figure 34: Fresh Water Ecotoxicity - Molecular methodology results for each scenario divided by process.

7.2.2.7 Marine aquatic ecotoxicity

Marine aquatic ecotoxicity (Figure 35) emerges as the primary vulnerability of this system. This high impact can largely be attributed to the incineration requirements for most of the generated waste, as well as the system's moderately high energy consumption. Incineration releases pollutants that can produce harmful compounds that can persist in the environment.

Additionally, the energy demand required to operate the system may indirectly contribute to ecotoxicity, as energy production involves fossil fuel combustion, which can release pollutants that ultimately accumulate in marine environments. These factors combined explain why marine ecotoxicity stands out as a critical area.

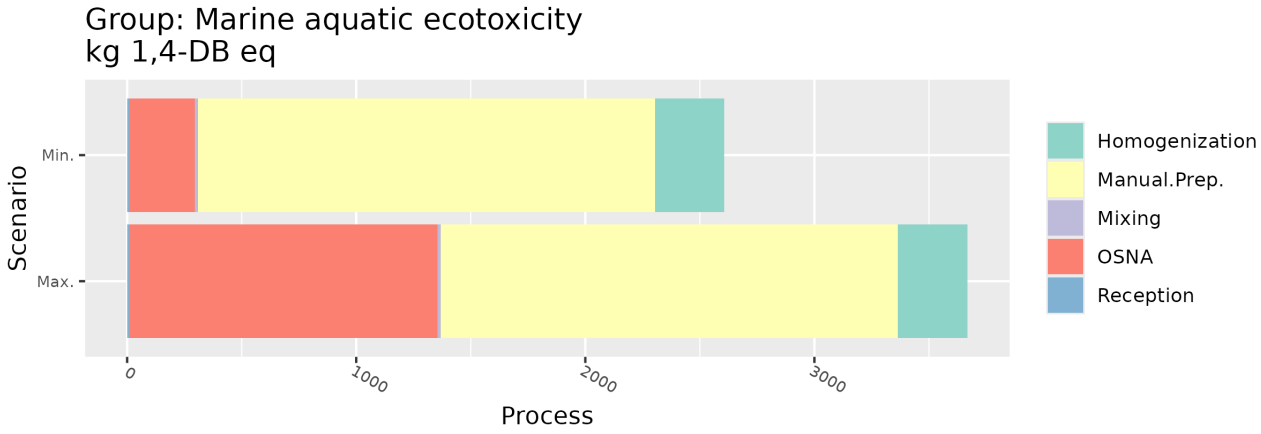

Figure 35: Marine aquatic ecotoxicity - Molecular methodology results for each scenario divided by process.

7.2.2.8 Terrestrial ecotoxicity

The moderate impact observed in terrestrial ecotoxicity (Figure 36) suggests that many of the pollutants generated by the system have a stronger tendency to persist and accumulate in aquatic environments rather than in soil or land ecosystems. This behavior indicates that these pollutants may be more water-soluble or prone to runoff, allowing them to migrate from terrestrial to aquatic systems where they pose greater long-term risks.

In particular, chemicals and waste products from the system might leach into water bodies or travel through drainage pathways, leading to more pronounced toxicity in freshwater and marine ecosystems compared to terrestrial ones.

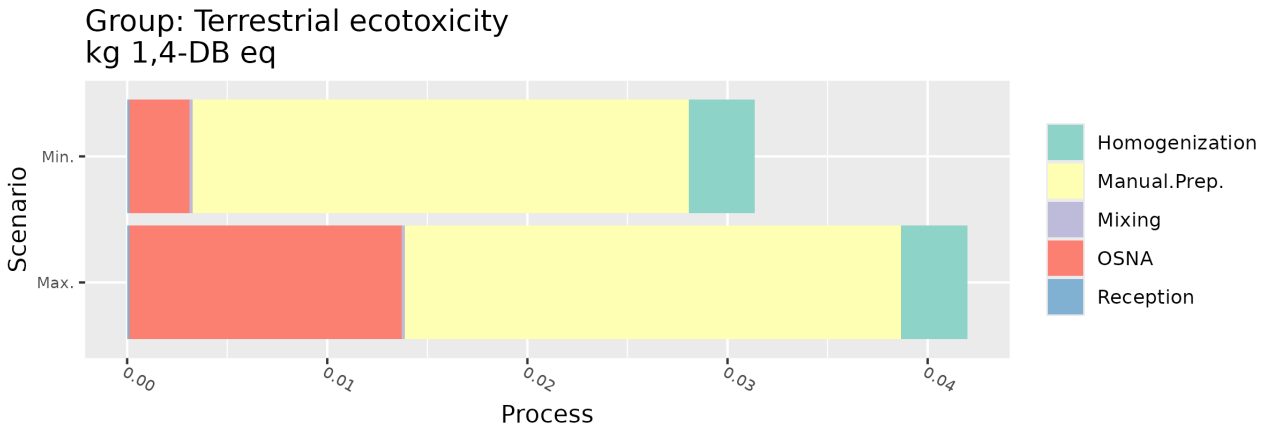

Figure 36: Terrestrial ecotoxicity - Molecular methodology results for each scenario divided by process.

7.2.2.9 Photochemical oxidation

The system demonstrates an exceptionally low impact on photochemical oxidation (Figure 37), an indicator of its limited contribution to smog formation and urban air pollution. This minimal effect is primarily due to the relatively low emissions of VOCs and NOx, which are the primary contributors to photochemical smog.

The low photochemical oxidation impact is largely attributed to the moderate energy requirements and transportation demands associated with the molecular methodology.

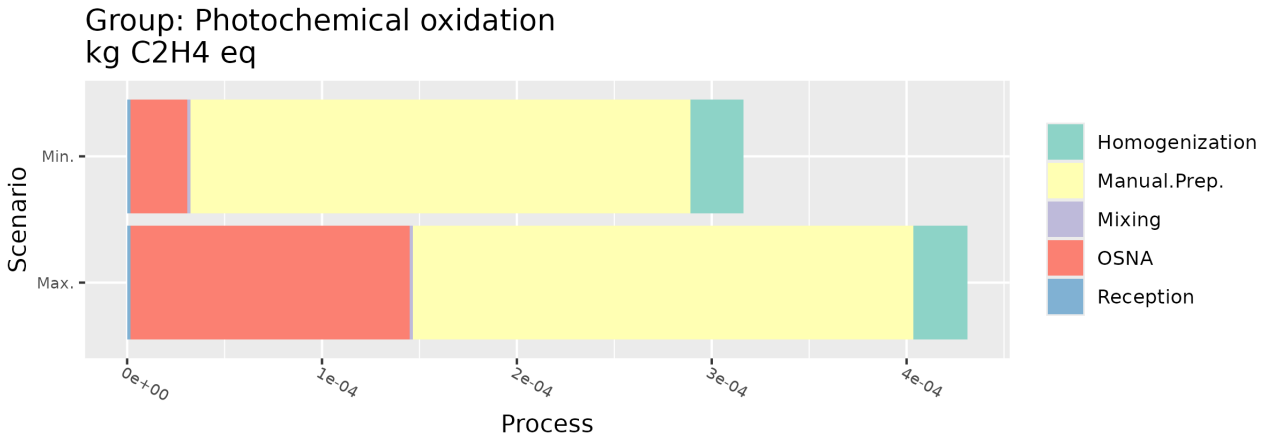

Figure 37: Photochemical oxidation - Molecular methodology results for each scenario divided by process.

7.2.2.10 Acidification

As with many other environmental impact categories, the primary contributor to acidification (Figure 38) is the "manual preparation" phase. This stage significantly impacts the system due to the energy-intensive process of manufacturing plastic garments, which requires substantial energy inputs for producing and processing plastics. Additionally, the overall electrical energy consumed during various processes within the system further contributes to the environmental footprint.

However, despite these contributing factors, the overall values across both scenarios remain relatively low.

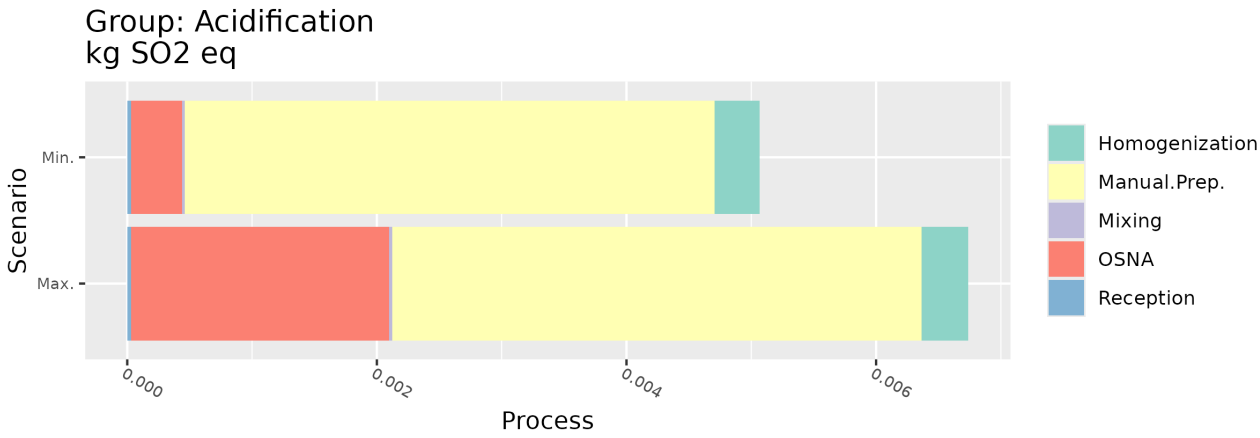

Figure 38: Acidification - Molecular methodology results for each scenario divided by process.

7.2.2.11 Eutrophication

The system exhibits a moderate impact on eutrophication (Figure 39), similar to its effect on acidification. This impact primarily stems from the production and disposal of single-use plastic garments. During manufacturing and disposal there can be nutrients release including nitrogen and phosphorus compounds. These nutrients can eventually make their way into water bodies, contributing to the eutrophication process.

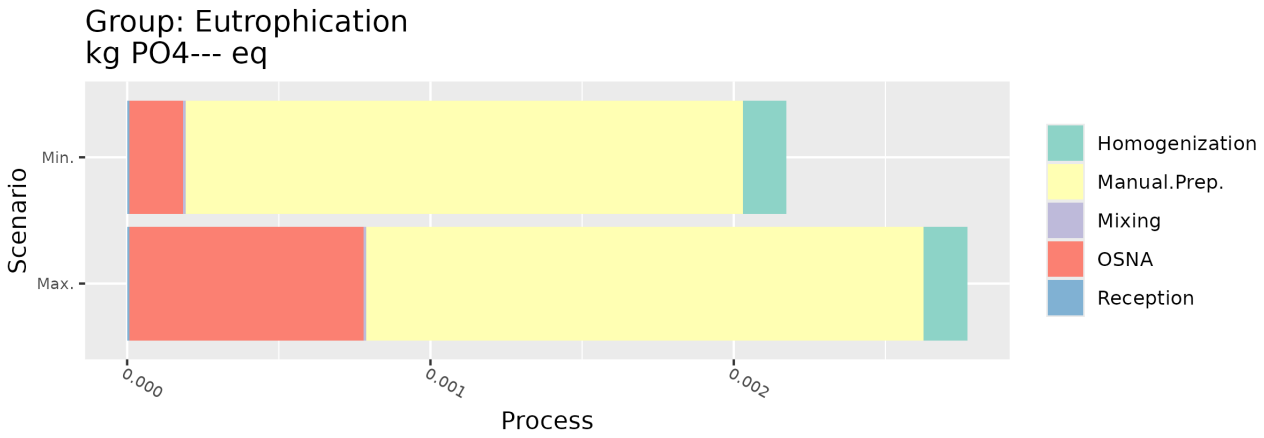

Figure 39: Eutrophication - Molecular methodology results for each scenario divided by process.

7.2.3 Comparison

When comparing histological and molecular methodologies (Figure 40), the environmental impact reveals a substantial difference, with the molecular method incurring only 8.9% of the environmental burden associated with histological techniques, on average. This considerable reduction in impact is evident across various environmental categories. On the high side of the spectrum, in the "Eutrophication" impact category, molecular methods exhibit 12.2% of the environmental impact generated by histological methods. On the lower side, in the "Terrestrial Ecotoxicity" category, the molecular method accounts for just 2.9% of the environmental load seen in histology.

It is also evident that, across various environmental impact indices, the histological methodology exhibits significantly higher variability compared to the molecular methodology. This increased variability can be attributed to the complexity and multi-step nature of histological workflows, which often involve a broad array of equipment with differing capabilities and energy requirements.

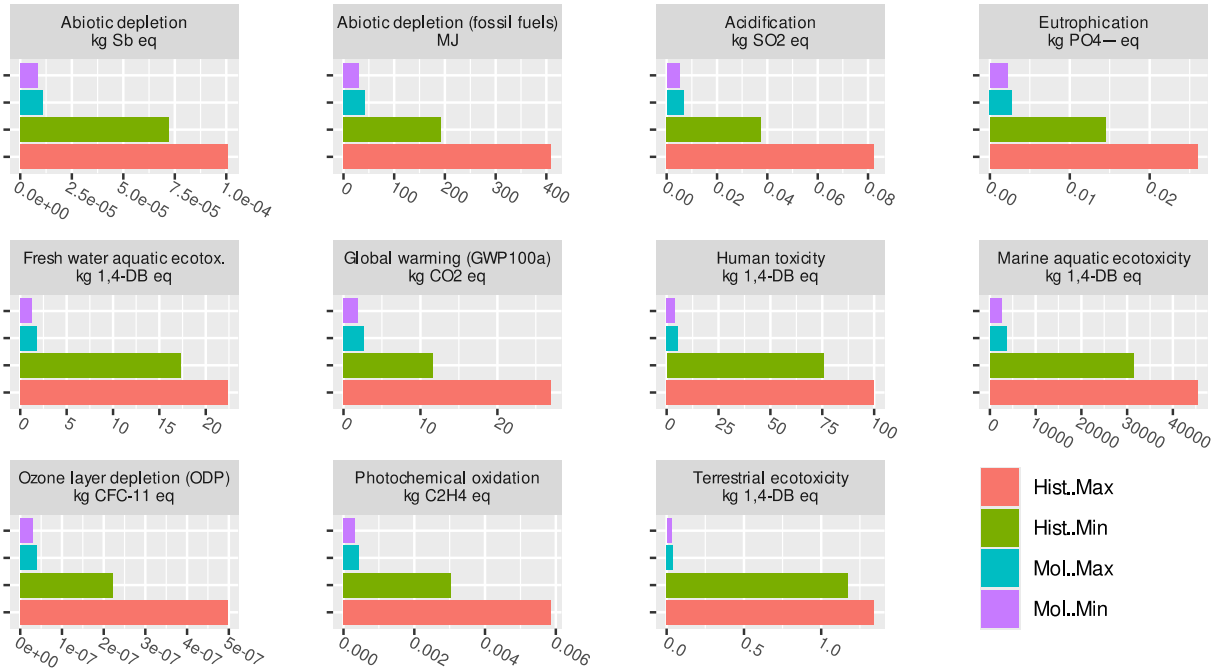

Figure 40: Methodology Comparison - Comparison between the impact of the two scenarios for each category between the two studied methodologies.

7.2.4 Uncertainty

The uncertainty analysis (Figure 41) shows a reasonable uncertainty in in most of the indexes whit a few of them having a moderately high standard deviation. In contrast we can notice that Terrestrial ecotoxicity exhibit an overwhelming variability in the simulation results. This give us some important insight about the main challenges that we face when applying LCA to a clinical laboratory setting and the processes that most contribute to each impact category.

In the background processes modeling we had two main data types that where not not present in LCA databases with a sufficient quality: Firstly, as previously mentioned, data regarding medical grade chemicals are totally lacking leading to estimation that intrinsically increase the uncertainty of the results. Secondly the there are a multitude of disposal methodologies that can be employed for biologically hazardous material but there are no standardized data reporting them. Few studies have been conducted regarding the various disposal methodology but most of them do no report the actual data and methodological approach making it impassible to reproduce or reliably use the results.

The lack of contextual data is particularly evident in the terrestrial ecotoxicity index, which is heavily influenced by long-term emissions and deposits of pollutants associated with the production and disposal of hazardous chemicals.

The high variability in this index reflects the compounded effects of these uncertainties, emphasizing the need for improved data collection and reporting standards in both medical-grade chemical use and waste disposal practices. Addressing these issues is critical for advancing the reliability and applicability of LCA in clinical laboratory settings.

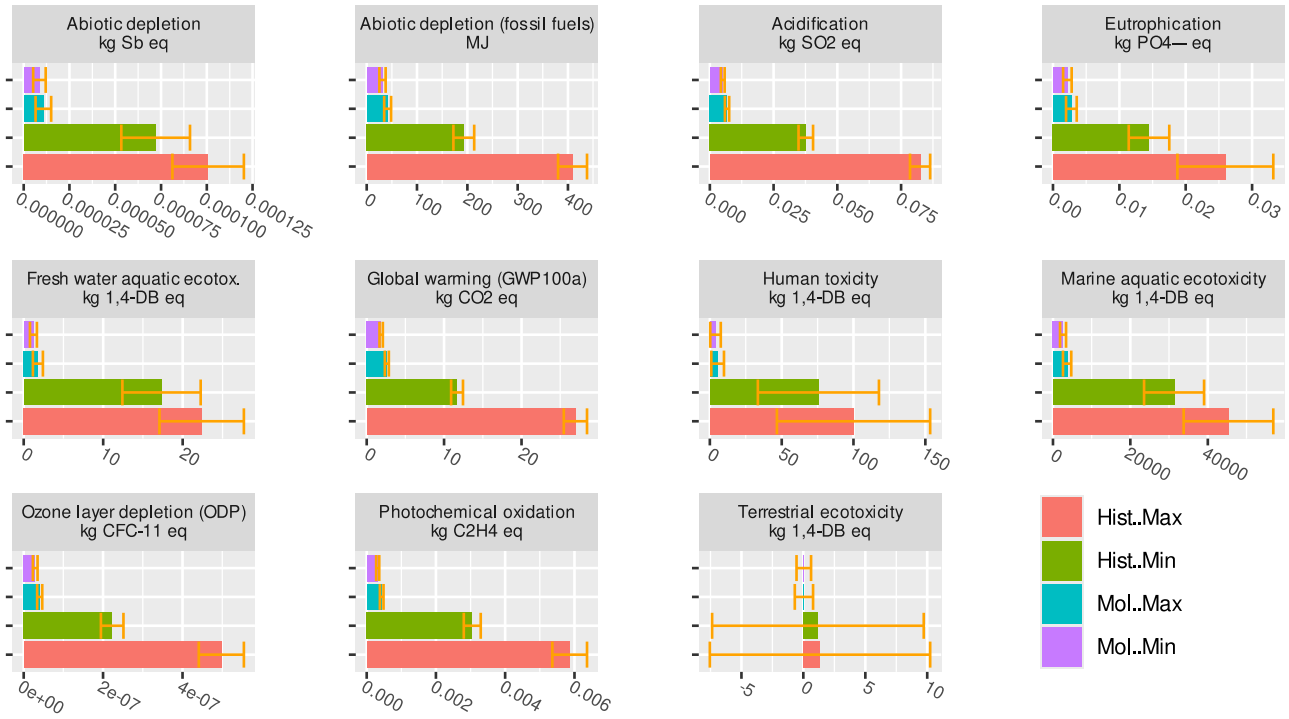

Figure 41: Uncertainty: Results for each diagnostic methodology and scenario with Standard Deviation visualization.

7.2.5 RNA Reagents

The sensitivity analysis conducted on the reagents containing RNA demonstrated that a **1000%** increase in the impact factor multiplier applied to these reagents resulted in only a negligible **0.5%** and **0.002%** increase in the overall environmental impact of the system respectively in the maximum and minimum efficiency scenarios. This outcome highlights that the RNA-based reagents, despite their potentially high individual impact factors, contribute minimally to the system's total impact.

The primary reason for this minimal contribution is the very small quantities of RNA-based reagents used in the procedures. While their environmental impact per unit might be significant, the overall effect is diluted due to their limited usage. This finding underscores the importance of considering both the impact intensity of individual components and their proportional use within the system when assessing their overall contribution to environmental burdens.

These findings highlight an important implications for optimizing the environmental impact of the system. The minimal contribution of RNA-based reagents to the overall impact, despite their potentially high individual impact factors, suggests that sustainability efforts should focus on other components, priority should be given to reagents or materials that are used in larger quantities and have a more substantial proportional effect on the total system impact, as these are likely to yield greater benefits in reducing the overall footprint.

8 Chapter : Interpretation

The analysis revealed a significant disparity between the two methodologies under consideration. Specifically, the molecular diagnostic method was found to have an environmental impact that averages only 10% of that associated with the histological method.

The reasons behind this stark contrast become apparent when analyzing the workflow and inventory requirements of each methodology. The histological methodology involves a complex sequence of procedures, each with its own demand for energy, water, and chemicals. Many of these procedures are highly energy-intensive, requiring constant heating, cooling, or other forms of controlled environments. Additionally, histology generates significant amounts of biologically hazardous waste, necessitating stringent waste handling and disposal protocols. From solvents to dyes and fixatives, each step in the histological process contributes to a cumulative environmental footprint that is difficult to mitigate.

In contrast, molecular methodologies, though not without their environmental challenges, rely on less extensive procedures that are generally more streamlined and require fewer hazardous chemicals. While some single-use plastic components are essential for molecular processes, the overall consumption of resources, especially reagents and packaging, is considerably lower. Thus, the molecular methodology has a comparatively lower environmental burden due to its simplified workflow and reduced need for energy-intensive operations and hazardous chemicals.

A primary environmental drawback of the molecular method is its dependence on custom-made, single-use plastic tools, which are essential for each stage of molecular analysis. These single-use plastics contribute significantly to the overall environmental footprint of the molecular method. Each tool is typically discarded after a single use, leading to a steady accumulation of biologically contaminated plastic waste that requires incineration. This reliance on disposable plastics also has indirect environmental impacts, as the production of these materials consume energy and resources, contributing to pollution and greenhouse gas emissions.

However, despite the environmental burden of these plastics, the molecular method generally requires fewer items, lower volumes of reagents, and less extensive packaging than the histological

method. In histology, a wide array of tools, consumables, and reagents is needed for multiple steps, including tissue fixation, staining, sectioning, and mounting. Each of these steps requires plastic containers, slides, pipettes, and other supplies, which are often wrapped in additional layers of plastic packaging for sterile handling. The cumulative environmental impact of this packaging, combined with the volume of reagents needed for each stage, results in a significant resource demand and waste generation in histological methodology.

One particularly interesting process to evaluate is "Manual preparation." This process, much like "Reception," is shared between both methodologies and is identical in its execution across the two models. However, its contribution to the total impact varies significantly depending on the methodology in use.

In the *Histological model*, "Manual preparation" contributes in an almost negligible manner to the total environmental burden. This suggests that the resources consumed and waste generated during this step are minimal compared to the other processes, making it appear as a relatively sustainable part of the process.

Conversely, in the *Molecular model*, "Manual preparation" emerges as the primary contributor to the overall environmental impact. This stark difference can be attributed to a single item: a disposable plastic apron. This apron, used during the preparation phase, accounts for the majority, by weight, of the waste generated by the molecular methodology. Its disposable nature, combined with the material's environmental footprint in terms of production, use, and disposal, significantly elevates the impact of this process.

This comparison underscores the importance of analyzing individual components within shared processes to identify disproportionate contributors to environmental burdens. Despite being the same procedure in both methodologies, the inclusion of a single high-impact item transforms "Manual preparation" into a critical focal point for sustainability improvements.

9 Chapter : Conclusion

This study aimed to implement a well-established and standardized methodology within a domain that has thus far seen limited application: surgical pathology laboratories. The objectives were twofold: first, to illustrate the potential and versatility of LCA methodology as a robust framework for environmental evaluation; and second, to underscore the importance of assessing clinical laboratory systems from an environmental perspective. By introducing LCA into this context, the research seeks to contribute to the broader discourse on sustainability in healthcare systems, highlighting the necessity of integrating environmental considerations into the operational and strategic planning of clinical laboratories.

With regard to the first objective, we demonstrated that is possible to apply the LCA methodology to multiple steps laboratory analysis in a reliable manner. However, a significant limitation became apparent during this process. LCA, by its design, relies on two types of data: foreground and background data. While foreground data can be directly collected from the specific systems under investigation, ensuring high accuracy and reliability, background data depends on per-existing analyses and datasets.

In the context of clinical laboratories, the novelty of environmental evaluations presents a clear challenge, as the field lacks a substantial body of prior studies and established datasets that could serve as a foundation for modeling background data. This scarcity directly impacts the precision of certain elements of the assessment, leading to a greater degree of approximation and, consequently, increased uncertainty in specific processes. Notable examples include the production and disposal of chemicals, where the limited availability of detailed and context-specific background data constrains the robustness of the analysis. This limitation underscores the need for further research and data collection to enhance predictive capabilities. At the same time, it suggests that with sustained effort and future research on this field, it is feasible to develop a cohesive and reliable data framework for applying LCA to laboratory systems.

Moreover, although this study focused on a specific laboratory setting, it demonstrated the feasibility of applying LCA methodology to surgical pathology laboratories in a broader range of environments. Since many of the procedures analyzed are performed by automated machinery

designed for specific, standardized tasks, a significant portion of the environmental data can be directly attributed to the machines themselves. This makes the environmental evaluation of each device largely context-independent. As a result, only a moderate number of factors remain location-specific, such as technician-dependent operations, environmental control needs (e.g., lighting, HVAC), local energy sources, and waste management systems. This underscores the critical role of specialized manufacturers in providing not only environmental impact data related to the production of laboratory equipment but also detailed inventories of their operational inputs and outputs. Such transparency would allow decision-makers to efficiently and reliably incorporate environmental considerations into the selection and design of analytical workflows.

Conversely, a detailed categorization of the diagnostic LCI could offer significant benefits to low-resource laboratories. By clearly distinguishing which components of the diagnostic process are context-dependent and which are not, such categorization would enable these facilities to make more informed and strategic decisions when selecting methodologies. This clarity would help laboratories identify which elements of an analytical workflow can be adapted or optimized within their specific constraints, ultimately supporting more sustainable and context-appropriate diagnostic practices.

Regarding the second objective, we achieved a satisfactory evaluation of the boundary conditions for the two diagnostic procedures. The analysis highlights that the primary contributors to the environmental impact, in both cases, are energy consumption and the incineration of biologically hazardous waste. These findings align closely with existing literature, which consistently identifies these factors as key drivers of environmental burdens in similar contexts.

Moreover, this study underscores the critical importance of a systematic and methodical approach to evaluation. By adhering to a structured analysis, we can effectively identify the most meaningful processes that warrant intervention, focusing our efforts on those that offer the greatest potential for improvement. This prevents us from being misled by processes that may initially appear to have a significant impact but, upon closer examination, either contribute minimally to the overall outcome or present substantial challenges to feasible improvements. This targeted approach ensures that resources and efforts are directed toward areas where they can have the most meaningful and practical impact, enhancing the efficiency and effectiveness of the decision-making process.

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