Deep Science Publishing
https://doi.org/10.70593/978-93-49307-52-0_2

Chapter 2

Essential laboratory equipment and tools: Functions, handling, and applications

Narendra Kumar[1], Keshawanand Tripathi[2], Yashdeep Srivastava[2*], Sarvesh Kumar Pandey[3]

[1]*School of Biotechnology and Bioengineering, Institute of Advanced Research, Gandhinagar, Gujarat, India.*

[2] *Department of Biotechnology, Invertis University, Bareilly, Uttar Pradesh, India.*

[3]*Department of Forensic Science, Invertis University, Bareilly, Uttar Pradesh, India.*

[*]Email: yashbubiotech@gmail.com

1. Compound Microscope:

1.1. Principle:

A microscope is an essential tool used to observe objects that are too small to be seen with the naked eye. It operates by magnifying these objects through a combination of lenses and illumination, revealing details and structures that would otherwise remain hidden. At its core, a microscope typically consists of several key components. The objective lenses, positioned beneath the stage, magnify the specimen, while the ocular lens, or eyepiece, further amplifies the image for observation. The total magnification is determined by the combined power of these lenses. The stage provides a stable platform for holding the specimen in place, allowing it to be easily manipulated for viewing. Light microscopes, the most common type, use visible light to illuminate the specimen. Bright-field microscopes provide basic magnification and contrast, while specialized techniques such as phase contrast and fluorescence microscopy enhance specific features or enable visualization of fluorescently labeled molecules (Thagela et al., 2018; Tiwari et al., 2022). In contrast, electron microscopes use beams of electrons instead of light to achieve much higher magnification and resolution. Microscopes are invaluable tools in scientific research, medicine, education, and industry, enabling discoveries across a wide range of disciplines and contributing to our understanding of the world at both the macroscopic and microscopic levels.

1.2. Parts of a compound microscope:

1.2.1. Objective Lenses: Typically, a compound microscope is equipped with multiple objective lenses, each offering different levels of magnification. These lenses are positioned on a rotating turret beneath the microscope's stage, allowing users to select the desired magnification level easily. Common magnifications range from 4X to 100X or higher, with higher magnifications reserved for specialized applications. The

magnification power of an objective lens refers to the factor by which it enlarges the specimen. For example, a 10X objective lens magnifies the specimen by ten times its actual size.

1.2.2. Ocular Lens: At the top of the body tube of the microscope, the ocular lens enlarges the image that the objective lens creates even more. Standard ocular lenses typically provide magnifications of 5X, 10X, or 15X. When combined with the magnification of the objective lens, the total magnification of the microscope is calculated by multiplying the magnification of the objective lens by the magnification of the ocular lens. For instance, if a 10X objective lens is paired with a 10X ocular lens, the total magnification would be 100X (10X objective magnification × 10X ocular magnification).

1.2.3. Condenser lens: Light focusing and direction onto the specimen is mostly dependent on the condenser lens of a microscope. It is positioned below the stage and gathers light from the microscope's illumination source to focus it onto the specimen for the best possible viewing. By adjusting the condenser's position and aperture, users can control the intensity and angle of the light, enhancing contrast and resolution. This precision in light manipulation is crucial for obtaining clear and detailed images of microscopic specimens. Overall, the condenser lens serves as a key component in maximizing the quality of microscopy imaging, facilitating accurate analysis and interpretation.

1.3. Common terms related with microscope:

1.3.1. Total Magnification: The product of the magnifications of the objective and ocular lenses of a compound microscope is the maximum magnification that can be achieved. Users can therefore change the overall magnification to meet their particular needs by changing the objective or ocular lenses. This flexibility enables researchers, clinicians, and educators to examine specimens at varying levels of detail, from macroscopic views to the microscopic realm.

1.3.2. Resolving Power: Resolving Power, essential for distinguishing between closely positioned points, is a critical aspect of microscopy. Much like a microbiologist, the microscope's condenser efficiently collects and focuses light onto the specimen slide, housing the organism under scrutiny.

1.3.3. Numerical Aperture: Numerical aperture, a fundamental parameter in microscopy, is determined by several factors including the wavelength of light, the angle of the lens aperture, and the refractive index of the surrounding medium. While adjusting these elements within conventional light microscope systems poses challenges, they significantly impact the numerical aperture and thus the resolving power of the microscope.

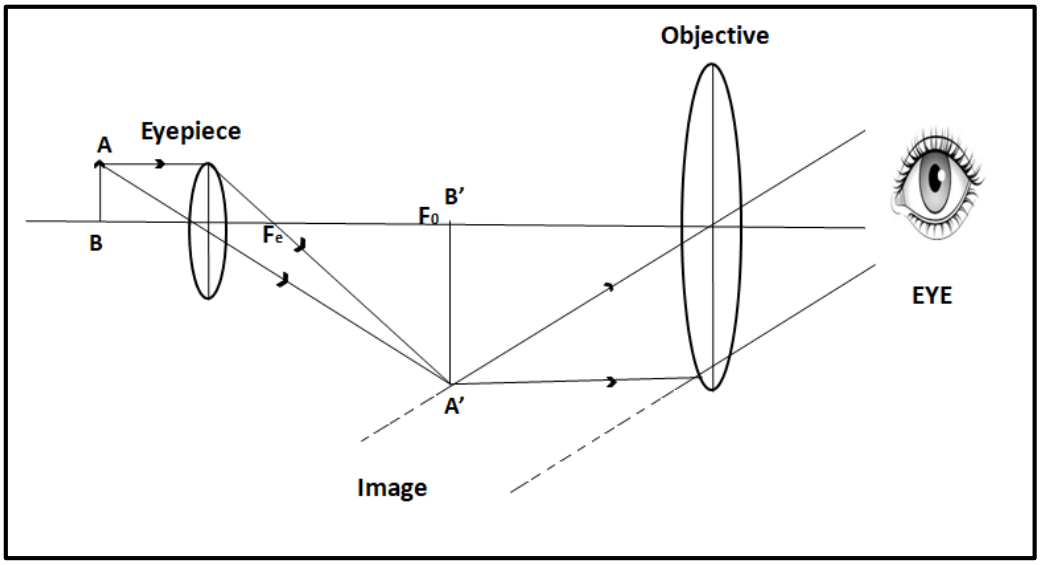

Figure 1: Ray diagram of Compound microscope

1.4. Effective Microscope Management:

1. To preserve the integrity of the glass lenses, do not contact them with your fingers.
2. Make sure to completely remove any oil residue from the surface of the oil immersion lens.
3. Do not modify any of the parts of the microscope.
4. Focus using the fine adjustment knob with 40X and 100X objectives after starting your observations with the lowest magnification objective (such as 4X or 10X).

1.5. Operating Procedures:

1. Place the slide onto the microscope stage.
2. Adjust the coarse knob to bring the stage close to the lens, and then carefully use the fine adjustment knob for precise focusing.
3. Make sure the organism of interest is centered in your frame of view after focusing on it using the lowest powered objective.
4. Position the next highest objective cautiously, viewing the slide from a different angle to prevent accidental contact.
5. Minor adjustments to the fine adjustment knob should suffice to bring the organism into clear focus.
6. After positioning the object correctly, increase magnification to the highest level using the 100X lens and adjust the light.

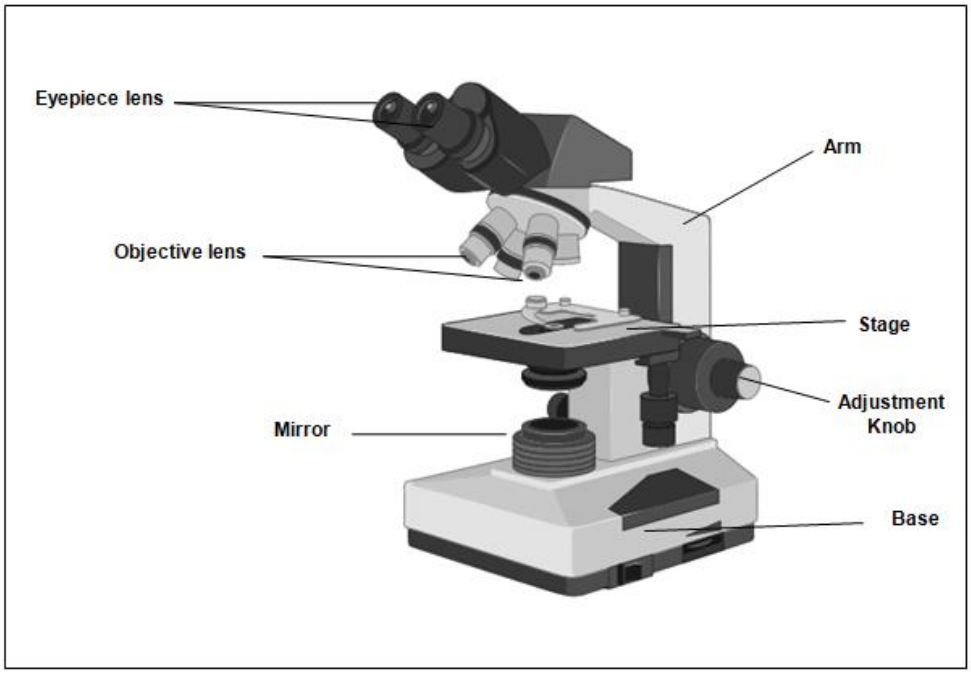

Figure 2: A compound microscope

1.6. Precautions:

1. **Handling**: Always handle the microscope with care, grasping it firmly with both hands when moving it to prevent accidental drops or damage.
2. **Cleaning**: Use lens paper and optical cleaning solution to gently wipe the lenses. Avoid using rough materials or excessive force, as this can scratch the lenses and degrade image quality.
3. **Light Source**: Be cautious when adjusting the light intensity to avoid overheating the specimen or damaging the microscope's bulb. Allow the microscope and bulb to cool down before handling or replacing the bulb.
4. **Specimen Preparation**: Ensure specimens are properly mounted and secured on slides to prevent movement or damage to the sample and microscope components.
5. **Magnification**: Start with the lowest magnification objective lens when focusing and then gradually increase to higher magnifications. Avoid forcing the focusing knobs to prevent damage to the internal mechanisms.
6. **Storage**: Store the microscope in a clean, dry environment, preferably covered to prevent dust accumulation on the lenses and other parts.
7. **Electrical Safety**: When using microscopes with electrical components (e.g., for illumination), ensure the power source is compatible with the microscope's requirements and follow electrical safety guidelines to prevent shocks or short circuits.

2. Autoclave:

2.1. Principle: Autoclaving is a crucial technique for ensuring the complete sterilization of media and glassware. This method relies on the use of moist heat sterilization. Understanding the functioning of an Autoclave involves grasping the fundamental principle at play: the temperature inside the autoclave is directly proportional to the pressure created within it. At normal atmospheric pressure, water reaches a boiling point of 100^{o}C.When steam is applied under pressure, the temperature inside the autoclave increases to 121^{o}C. Moist heat is believed to eliminate microorganisms by inducing the coagulation of vital proteins.

2.2. Standard procedure for operating the autoclave:

2.2.1. Preparation and Loading of Materials:

1. Fill the containers only halfway with water. Operate the instruments with precision.
2. Make sure to loosen the caps or use vented closures.
3. Remember to place bags of biological waste in pans to prevent any spills.
4. Make sure to leave enough space between items to allow for proper steam circulation.

2.2.2. Taking off the load:

1. Make sure to verify that the chamber pressure is at zero.
2. Remember to stand behind the autoclave lid when opening it.
3. Open the lid slightly. Be cautious of a sudden release of steam.
4. After completing the exhaust cycle, it is important to open the autoclave door and let the liquids cool for duration of 20 minutes before they can be safely removed.

2.3. Precautions:

1. **Read the Manual**: Familiarize yourself with the manufacturer's instructions and guidelines for the specific autoclave model you are using. Follow all operational and safety instructions meticulously.
2. **Proper Loading**: Ensure items to be sterilized are loaded correctly according to the autoclave's capacity and arrangement guidelines. Leave adequate space between items for steam circulation.
3. **Use Suitable Containers**: Use autoclave-safe containers and pouches designed for sterilization to prevent damage to the autoclave and ensure proper sterilization of contents.
4. **Monitor Temperature and Pressure**: Always monitor the temperature and pressure gauges during the sterilization process. Ensure that the autoclave reaches and maintains the appropriate temperature and pressure levels for the specified duration.

5. **Ventilation**: Allow proper ventilation and cooling before opening the autoclave after the sterilization cycle completes to prevent exposure to hot steam and potential burns.
6. **Personal Protective Equipment (PPE)**: Wear appropriate PPE, such as heat-resistant gloves and safety goggles, when handling hot containers or opening the autoclave to protect against steam burns and other hazards.
7. **Regular Maintenance**: Schedule regular maintenance and calibration of the autoclave according to manufacturer recommendations to ensure proper functionality and safety.
8. **Emergency Procedures**: Familiarize yourself with emergency procedures, such as how to safely abort a cycle or handle malfunctions. Have emergency contact information readily available.
9. **Cooling**: Allow sufficient cooling time for sterilized items before handling to avoid burns and ensure effectiveness of the sterilization process.

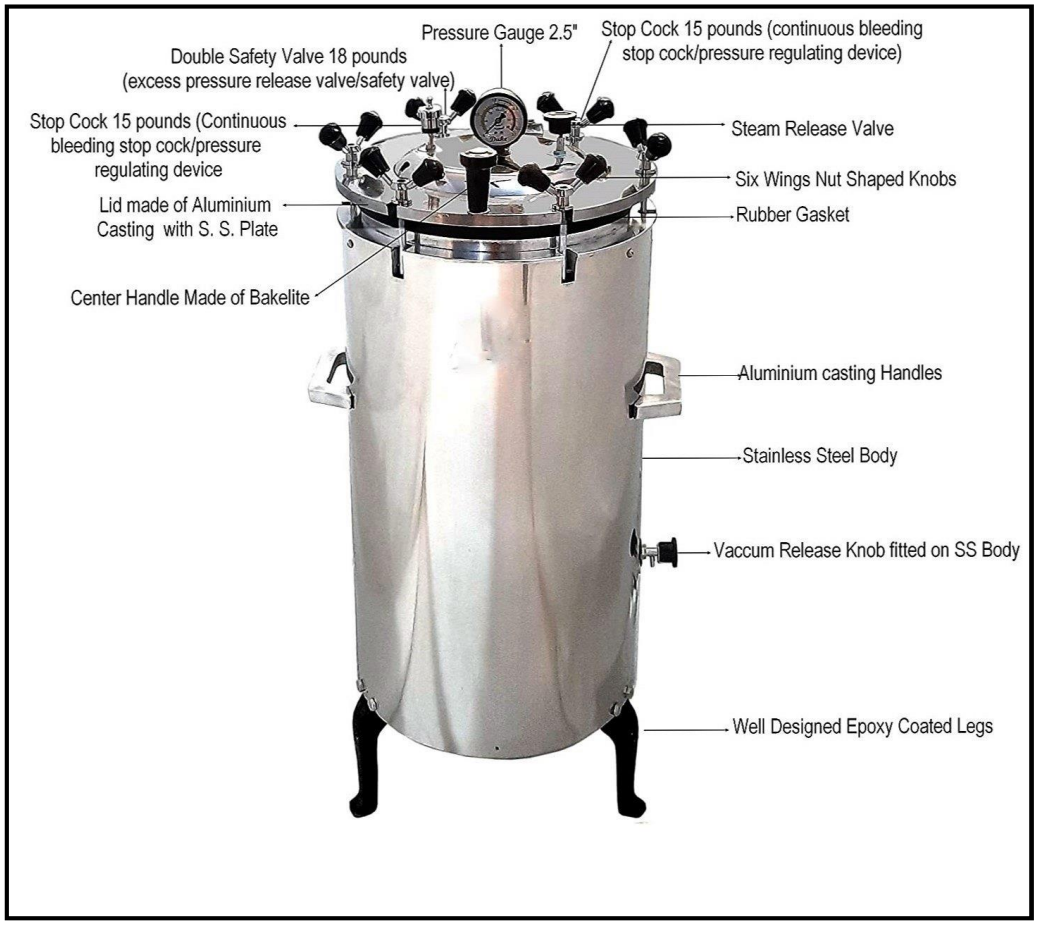

Figure 3: Working model of Autoclave

3. Incubator:

3.1. Principle: An incubator serves as a vital tool for cultivating and maintaining microbiological or animal cell cultures in an optimal temperature and environment. These chambers are essential for cell culture, microbiology, and molecular biology, accommodating both bacterial and eukaryotic cells. Much like a diligent scientist, the incubator meticulously regulates and sustains the ideal temperature, humidity, and atmospheric conditions, including levels of carbon dioxide (CO_2) and oxygen (Tripathi et al., 2018).

3.2. Operation:

1. Set your preferred temperature using the temperature control knob.
2. Turn the knob right to increase the chamber temperature and left to decrease it.
3. To activate the incubator, switch on the power and adjust the temperature to your desired level.
4. The heater lamp will illuminate and remain on until the chamber reaches the set temperature.
5. Allow at least 60 minutes for the chamber to equilibrate to the desired temperature when starting from a cold state.
6. Allocate a minimum of 15 minutes for the re-equilibration process when adjusting temperatures.

Figure 4: **A.** CO_2 and **B.** BOD incubator.

3.3. Precautions:

1. **Temperature Settings**: Set the temperature according to the specific requirements of your experiment or culture. Ensure the temperature is stable and regularly monitored using built-in thermometers or external temperature probes.
2. **Humidity Control**: If your experiment requires specific humidity levels, ensure the incubator's humidity settings are adjusted accordingly. Use water trays or humidity control features provided by the manufacturer.
3. **Sterility Maintenance**: Keep the interior of the incubator clean and sterilized. Regularly clean shelves, walls, and vents using appropriate disinfectants to prevent contamination of cultures.
4. **Avoid Overcrowding**: Do not overcrowd the incubator with too many cultures or experiments. Ensure adequate space between samples for proper air circulation and heat distribution.
5. **Routine Monitoring**: Regularly monitor the cultures or experiments inside the incubator for signs of contamination, condensation, or abnormal growth patterns. Remove any contaminated samples promptly to prevent spreading.
6. **Proper Ventilation**: Ensure adequate ventilation around the incubator to prevent overheating and allow proper functioning of internal fans or airflow mechanisms.
7. **Power Supply**: Ensure the incubator is connected to a stable power supply with the appropriate voltage and current rating as specified by the manufacturer. Use surge protectors or voltage stabilizers if necessary to protect the equipment from power fluctuations.
8. **Access and Handling**: Handle cultures and samples inside the incubator carefully to avoid sudden temperature changes or spills. Open and close the door gently to minimize disruption to the internal environment.
9. **Emergency Procedures**: Familiarize yourself with emergency procedures provided by the manufacturer, such as how to safely shut down the incubator in case of malfunction or power outage. Have emergency contact information readily available.
10. **Regular Maintenance**: To guarantee longevity and optimum performance, clean, calibrate, and service the incubator according to the manufacturer's suggested maintenance schedule.

4. Laminar air flow

4.1. Principle: The use of laminar air flow to generate a sterile environment for microbial work. Through the use of a specialized filter called HEPA (High Efficiency Particulates Air), this device ensures that the air passing through it remains free from any microbes, dust particles, and fungi, thus creating a sterile environment. **Uses:** Nutrient culture plate preparation, slant preparation, culture inoculation, isolation, streaking, and other microbial and fungal tasks.

Figure 5: Laminar air flow

4.2. Precautions:

1. **Sterile Techniques**: Always use sterile techniques when working inside the laminar airflow cabinet. This includes wearing sterile gloves and using sterile instruments and materials to prevent contamination of the workspace.
2. **Proper Setup**: Ensure the laminar airflow cabinet is properly set up and functioning before use. Check that the HEPA filters are clean and operational to maintain the required airflow and sterility.
3. **Avoid Turbulence**: Minimize movements and disruptions that can disturb the laminar airflow. Place materials and equipment gently into the cabinet to avoid creating turbulence that could compromise the sterile environment.
4. **Routine Cleaning**: Regularly clean and disinfect the interior surfaces of the laminar airflow cabinet. Use appropriate disinfectants recommended by the manufacturer to prevent microbial growth and maintain sterility.
5. **Limit Access**: Limit access to the laminar airflow cabinet to authorized personnel only. Ensure that users are trained in proper procedures and understand the importance of maintaining a sterile environment.
6. **Monitoring Airflow**: Monitor the airflow velocity and direction regularly using built-in airflow indicators or anemometers. Ensure that the laminar airflow is uniform and directed away from the operator to protect both the samples and the user.
7. **Safety Equipment**: Ensure that safety equipment, such as gloves, lab coats, and safety goggles, is worn when handling materials inside the laminar airflow cabinet to protect against spills and splashes.

8. **Emergency Procedures**: Familiarize yourself with emergency procedures, such as what to do in case of power failure or equipment malfunction. Have emergency contact information readily available
9. **Regular Maintenance**: For the laminar airflow cabinet to operate as efficiently and safely as possible, replace the filters, verify airflow performance, and perform other maintenance as advised by the manufacturer.

5. Ultraviolet-visible (UV-Vis) Spectrophotometer

5.1. Principle:

UV-visible (UV-Vis) spectroscopy is a widely used analytical method used to measure the absorption or transmission of particular UV or visible light wavelengths with respect to a reference or blank sample. This characteristic, which is determined by the makeup of the sample, makes its contents and concentrations visible. Shorter wavelengths possess higher energy due to the inverse relationship between energy and wavelength in light. Various substances can absorb light at different wavelengths. For instance, ultraviolet (UV) light is absorbed at shorter wavelengths than visible light, typically up to approximately 100 nm. UV-VIS spectroscopy precisely identifies the wavelengths corresponding to maximum absorbance, facilitating the analysis and identification of different chemicals (Tripathi et al., 2013a, b).

5.2. Working:

The sample is placed in the sample compartment so that the monochromatic beam can pass through it in UV-Vis spectroscopy. For absorbance measurements, liquid samples are typically put in a cuvette with a predetermined pathlength. These cuvettes, which are usually composed of glass, quartz, or plastic, help transmit visible or ultraviolet light. While plastic cuvettes might only transmit visible light, conventional quartz cuvettes with a 10 mm pathlength maximize UV transmission. Different cuvettes cater to diverse applications, with some designed for small liquid volumes and others for longer pathlengths to analyze very dilute samples. For solid samples, transmission measurements are straightforward, while accessories can enhance capabilities for complex measurements like diffuse reflectance or transmission. Fiber optics provides a versatile solution for analyzing large, hazardous, or inaccessible samples. Utilizing a fiber-optic probe, UV-Vis light can be transmitted from the spectrophotometer to analyze solutions externally. Alternatively, a fiber-optic device can measure light reflectance, fluorescence, or transmission through solid samples. These methods extend UV-Vis spectroscopy's applicability across a broad spectrum of sample types and conditions.

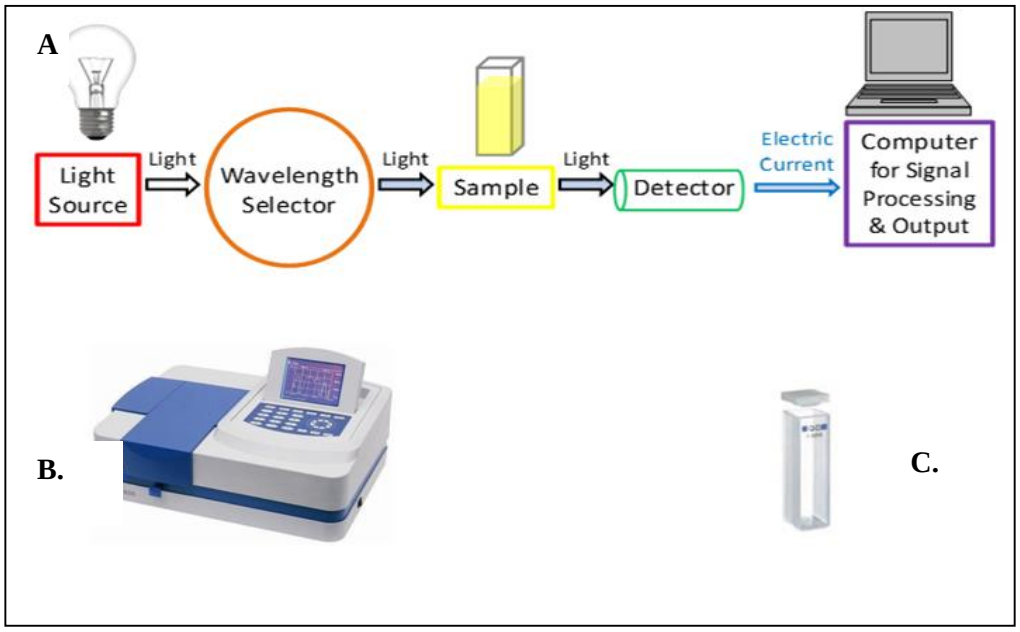

Figure: 6 A. Working Diagram of UV-VIS spectrophotometer. **B.** UV-VIS spectrophotometer. **C**. Cuvette

5.3. Precautions:

1. **UV Protection**: Wear appropriate personal protective equipment (PPE), such as UV-blocking safety goggles and gloves, when operating the UV-Vis spectrophotometer. UV radiation can be harmful to eyes and skin.
2. **Sample Preparation**: Ensure samples are prepared correctly according to the spectrophotometer's requirements. Use clean and transparent cuvettes or sample holders to avoid interference with measurements.
3. **Calibration**: Regularly calibrate the spectrophotometer using certified reference materials or calibration standards provided by the manufacturer. This ensures accurate readings and reliable performance.
4. **Avoid Contamination**: Keep the sample compartment and optical surfaces clean to prevent contamination that can affect measurements. Use lint-free wipes and appropriate cleaning solutions recommended by the manufacturer.
5. **Power Supply**: Ensure the spectrophotometer is connected to a stable power supply with the correct voltage and frequency as specified by the manufacturer. Use surge protectors if necessary to protect the equipment from power fluctuations.
6. **Wavelength Range**: Operate within the specified wavelength range of the UV-Vis spectrophotometer. Avoid using wavelengths outside the instrument's capabilities, as this can damage the optics and affect measurements.
7. **Temperature Control**: Maintain stable ambient temperature around the spectrophotometer to prevent thermal fluctuations that can affect readings. Use a temperature-controlled environment if necessary for sensitive measurements.

8. **Ventilation**: Ensure adequate ventilation around the spectrophotometer to prevent overheating during prolonged use. Avoid blocking ventilation ports or placing the instrument in confined spaces.
9. **Data Handling**: Handle data and results with care to ensure accuracy and traceability. Record all experimental parameters, including wavelengths, absorbance values, and conditions, for future reference and reproducibility.
10. **Routine Maintenance**: Follow the manufacturer's recommended maintenance schedule for cleaning, recalibration, and servicing of the UV-Vis spectrophotometer. This helps ensure optimal performance and extends the lifespan of the instrument.

6. References:

1. Rao, S.R., & Ghosh, T. (2011). Good laboratory practices in biological research. *Journal of Scientific Research, 55*(2), 112-118.
2. Hughes, G.J., & Smith, J.D. (2010). Basic laboratory skills in life sciences: An overview of essential techniques. *Biotechnology Advances, 28*(3), 325-337.
3. Myers, J.R. (2015). Best practices for laboratory safety and hygiene in microbiology. *Applied and Environmental Microbiology, 81*(19), 6475-6483.
4. Martins, J.M., & Silva, L.R. (2014). Laboratory organization and standard operating procedures for beginners. *Nature Protocols, 9*(7), 1113-1121.
5. Rittman, B.E., & McCarty, P.L. (2012). Laboratory methods for biological research in environmental sciences. *Environmental Science & Technology, 46*(12), 6545-6552
6. Tiwari, P., Srivastava, Y., & Kumar, V. (2022). Antimicrobial peptides as effective agents against drug-resistant pathogens. *Antimicrobial Resistance: Underlying Mechanisms and Therapeutic Approaches*, 289-322.
7. Thagela, P., Yadav, R. K., Tripathi, K., Singh, P. K., Ahmad, A., Dahuja, A., & Abraham, G. (2018). Salinity induced changes in the chloroplast proteome of the aquatic pteridophyte Azolla microphylla. *Symbiosis*, *75*, 61-67.
8. Tripathi, K., Sharma, N. K., Rai, V., & Rai, A. K. (2013). Low cellular P-quota and poor metabolic adaptations of the freshwater cyanobacterium Anabaena fertilissima Rao during Pi-limitation. *Antonie van Leeuwenhoek*, *103*, 277-291.
9. Tripathi, K., Sharma, N. K., Kageyama, H., Takabe, T., & Rai, A. K. (2013). Physiological, biochemical and molecular responses of the halophilic cyanobacterium Aphanothece halophytica to Pi-deficiency. *European Journal of Phycology*, *48*(4), 461-473.
10. Tripathi, K., Kumar, N., & Abraham, G. (Eds.). (2018). *The role of photosynthetic microbes in agriculture and industry*. Nova Science Publisher's, USA