



**Table 6: Types of microbiological examination<sup>1-4</sup>**

Type	Mechanism	Resolution limit (maximum magnification)	Type of causative microorganism		Considerations for use
			Planktonic	Biofilm	
<b>Light microscopy</b>	Visible light	0.2 µm (1500x)	✓	✓	<ul style="list-style-type: none"> <li>Primarily used on isolated cultures or sections of tissue</li> <li>Gram stain used to establish presumptive species identification</li> <li>Impossible to obtain definitive identification of microbial species</li> <li>Low-cost and readily available</li> </ul>
<b>Fluorescence microscopy (FISH)</b>	Ultraviolet light	0.1 µm (2000x)	✓	✓	<ul style="list-style-type: none"> <li>Species can be identified and their relative locations mapped with fluorescent dyes/labels</li> <li>Only fluorescent structures can be observed</li> <li>Use is limited to microbial cell suspensions and thin tissue sections</li> <li>Cost of dyes and probes is a limitation</li> </ul>
<b>Confocal laser scanning microscopy (CLSM)</b>	Laser beam coupled to a light microscope	0.1 µm (2000x)	✓	✓	<ul style="list-style-type: none"> <li>Species can be identified and their relative locations mapped with fluorescent dyes/labels</li> <li>Tissue blocks can be examined and images obtained at regular depths can be reconstructed to generate 2D or 3D structure of whole specimen</li> <li>Only fluorescent structures are observed</li> <li>Fluorescence decays relatively quickly</li> <li>Cost of equipment, dyes, probes, and technical support is a limitation</li> </ul>
<b>Scanning electron microscopy (SEM)</b>	Electrons beamed onto specimen from an angle and deflected electrons collected	10 µm (500,000x)	✓	✓	<ul style="list-style-type: none"> <li>Cannot examine living material</li> <li>Minimal time required for sample preparation</li> <li>Images of the surface layers of specimens provide insight into 3D structure</li> <li>Dehydration of samples may cause changes</li> <li>Cost of equipment and technical support is a limitation</li> </ul>
<b>Transmission electron microscopy (TEM)</b>	Electrons beamed through a thin section of specimen	0.2 µm (5,000,000x)	✓	✓	<ul style="list-style-type: none"> <li>Images provide detailed information on internal cellular structures or organisms</li> <li>Cannot examine living material</li> <li>Specimen preparation is lengthy, and may introduce artefacts</li> <li>Cost of equipment and technical support is a limitation</li> </ul>
<b>Polymerase chain reaction (PCR)</b>	Amplifies specific regions of DNA	0.1 and 10 kilobase pairs	✓		<ul style="list-style-type: none"> <li>Can confirm genes of interest from bacteria, toxins, viruses and other microorganisms</li> <li>Rapid and highly specific</li> <li>Identifies non-cultivable or slow growing microorganisms such as mycobacteria, anaerobes, or viruses</li> </ul>

## Table 06 References

1. Davidson MW. *Microscopy U*. 2016; Available from: <http://www.microscopyu.com/>.
2. Wilson SM and Antony B, Preparation of plant cells for transmission electron microscopy to optimize immunogold labeling of carbohydrate and protein epitopes, Table 1: Advantages and limitations of different microscopy techniques. *Nat Protoc*, 2012. 7: p. 1716-27.
3. Edward-Jones G, *Collection, transport, and laboratory processing of wound, tissue and bone samples*, in *Essential microbiology for wound care*, Edward-Jones V, Editor. 2016, University press: Oxford. p. 33-51.
4. Achinas S, Yska SK, Charalampogiannis N, Krooneman J, and Euverink GJW, A technological understanding of biofilm detection techniques: A review. *Materials* (Basel, Switzerland), 2020. 13(14): p. 3147.