Application Note

Clinical Laboratory Testing of Sputum

ZEISS Primo Star iLED

ZEISS

Seeing beyond

Author: Dr. Silvia Zenner-Gellrich
Carl Zeiss Microscopy GmbH, Germany
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In 1882, Robert Koch discovered *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis. He used optics by ZEISS for his work. Even today, laboratory microscopes from ZEISS still play an important role in the battle against infectious diseases.

Infectious diseases are a risk factor

Infectious diseases are pathogen-related diseases of the body, which show different courses of disease depending on the constitution of the person concerned. In pharmaceutical and biomedical research, drugs have been developed to fight against a variety of pathogens. Despite possible treatment with antituberculotics, the bacterial infectious disease tuberculosis, for example, is among the top 10 leading causes of death (1). Tuberculosis is mainly spread by droplet infection and attacks the lungs first (Figure 1).

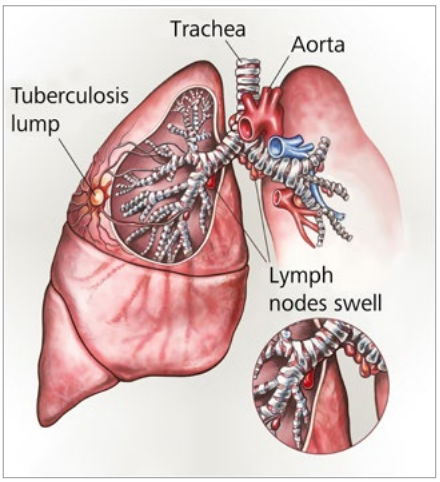


Figure 1 *Pulmonary tuberculosis, caused by droplet infection*

The development of multi-resistant strains and coinfection with the HIV virus complicate the course of the disease, but tuberculosis can be cured if detected early and treated consistently. A quick assessment is an essential success factor: the earlier treatment can begin for a tuberculosis patient, the greater the chance of a full recovery.

Tried-and-tested standard: sputum testing under the microscope

Sputum testing under the microscope is the gold standard and common practice in medical laboratories. A smear of sputum is applied to a microscope slide, fixed and stained with dye. The sample is not provided with a cover glass.
Due to the small size of the pathogens stained according to Ziehl-Neelsen, the transmitted light brightfield method is often used with 100× objectives. At this objective magnification rate, the visible object field is correspondingly small.
As a result, scanning of the specimen is relatively time-consuming. Use of the fluorescence contrast method can accelerate sputum testing. Fluorescence excitation causes the specifically stained mycobacteria to light up a yellow-greenish color against a dark background. Due to this fact and the use of a 40× objective with larger object field, the detection of the pathogens is about four times faster. The sensitivity of the pathogen detection can also be increased by up to 10 %.

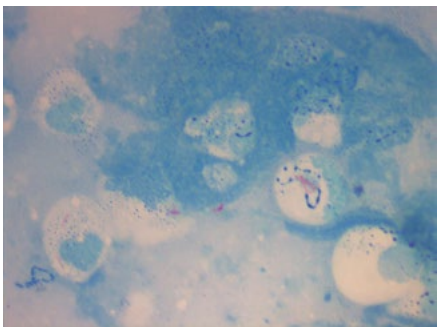


Figure 2 *Mycobacterium tuberculosis, Ziehl-Neelsen stain: the purple-stained mycobacteria become visible in the 100× oil objective. Courtesy of Dr. H. Hoffmann, WHO – Supranational Reference Laboratory IML Gauting, Germany*

Dyes make acid-resistant mycobacteria visible

In order to detect tuberculosis pathogens easily, quickly and reliably and to benefit from the advantages of fluorescence microscopy, the pathogen is usually stained with the dye Auramine O. The sputum is spread evenly on a marked microscope slide for this purpose. The air-dried smear is fixed with heat. The dye Auramine O, which is now applied, settles in the cell walls of the mycobacteria. The staining time is around 20 minutes. An acid-alcohol mixture is then applied for discoloration. Non-acid-resistant bacteria fade, and only the acid-resistant mycobacteria remain stained. The acid-alcohol mixture is then rinsed off using distilled water. Various dyes such as methylene blue, potassium permanganate or ink can be used for counterstaining. After air-drying the microscope slides, the tubercle bacilli can then be quickly identified under the fluorescence microscope as luminous rods against a dark background.

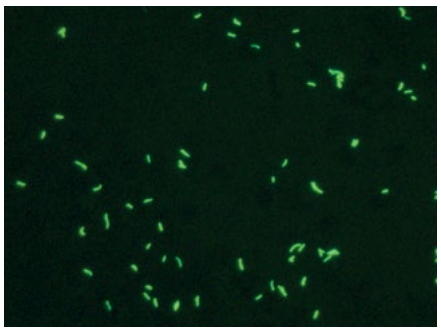


Figure 3 *After staining with Auramine O, the acid-resistant mycobacteria can be seen in the fluorescence microscope with 40× objective as greenish glowing rods against a dark background. Courtesy of Dr. H. Hoffmann, WHO – Supranational Reference Laboratory IML Gauting, Germany*

Figure 5 *Staining of smears for tuberculosis diagnosis with fluorescence microscopy*

If no acid-resistant rods are found in at least 100 to 300 object fields, there is no pathogen infection. Up to 50 preparations can be processed per day with the help of fast detection by fluorescence microscopy.

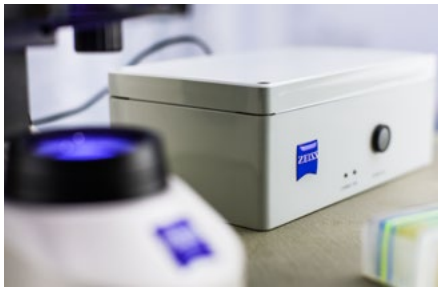
Brightfield and fluorescence: ZEISS Primo Star iLED combines both

Primo Star iLED is an LED-based fluorescence microscope. Clever details enable easy handling and flexible use of the device:

- With just a few hand movements, the device can be used as a brightfield microscope and as a fluorescence microscope.
- Use of LEDs is not only economically efficient: compared to mercury vapor lamps as fluorescent light sources, LEDs offer many advantages in use. For operation in the field and for regions with a fluctuating power supply, a battery pack was developed that allows the fluorescence microscope to be operated for several hours without an electricity supply.
- The special eyecups darken the ambient light almost completely, meaning there is no need to work in the darkroom.

Figure 4 *Primo Star iLED: switch from brightfield methods to fluorescence contrast in just a few steps*

Optional accessories:



- Battery supply unit (415500-1814-000)
- Transport and storage case

(415500-1827-000)

Summary

Efficient testing with subsequent treatment of those affected is essential for the fight against tuberculosis. For this reason, the WHO recommends sputum testing using fluorescence microscopy. Since the mycobacteria light up in fluorescence contrast against a dark background, and you can use a 40× objective at the same time, this method can accelerate the examination of smears.

"Primo Star iLED is the long-awaited answer to the three most pressing problems of tuberculosis microscopy in our partner countries.

1. *The microscope offers fluorescence and transmitted light brightfield microscopy in one, but does not require changing or adjusting lamps or charcoal burners. You can switch between fluorescent and brightfield illumination with a single flip switch. When operating the microscope becomes that easy, training microscope users becomes much easier.*
2. *In addition to the low purchase price, Primo Star iLED is impressive because it reduces the running costs of fluorescence microscopy from over a dollar per hour to less than half a cent. This was achieved by replacing the expensive mercury vapor lamp with inexpensive light emitting diodes with a long life. This makes diagnostically better fluorescence microscopy affordable for everyone.*
3. *Primo Star iLED requires hardly any power and can be powered by batteries for many hours. In this way, tuberculosis microscopy can also be offered in areas where there is no reliable power supply. Primo Star iLED is a jack-of-all-trades with a handy, robust shape and attractive design. It will certainly become an integral part of tuberculosis diagnostics in our partner countries."*

Dr. med. Harald Hoffmann

Head of the Institute of Microbiology and Laboratory Medicine in Gauting (Munich); WHO Supranational Reference Laboratory of Tuberculosis

Reference:

[1] WHO Global Tuberculosis Report 2019.
https://www.who.int/tb/publications/global_report/tb19_Exec_Sum_12Nov2019.pdf?ua=1

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Carl Zeiss Microscopy GmbH
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/primostar