



English

Leja pre-stained SpermBlue slides for morphology analysis

Producer: Leja Products B.V., Luzernestraat 10, 2153 GN Nieuw-Vennep, the Netherlands.

Distribution: see www.leja.nl

Intended use: to assess the morphological parameters of cells such as spermatozoa. The stained cells can be assessed with the help of an automated system (i.e. CASA) or manually.

Principle of the device: when a small volume of cells / semen is deposited on a pre-stained slide, the fixative-stain film dissolves in fluid/seminal plasma and stains (sperm) cells.

Description: Leja pre-stained SpermBlue slides are produced in the Netherlands by coating a regular slide with SpermBlue™ solution. The slides have a marking area for noting patient ID for future reference.

Preparations before use: remove Leja pre-stained morphology slide from packaging and take care not to touch the coated area. Make sure the coated area is undamaged. Access to an incubator at 55°C / 131F, or an ethanol flame. Use semen after liquefaction or (sperm) sample in a culture medium.

Using the Leja pre-stained SpermBlue slides: pipette 1µl of sample on the middle of the slide. Wait 30 seconds. Carefully apply a 22 x 22 mm cover slip ensuring that no air bubbles form. Wait 5 seconds. Using tweezers or a pencil, push gently on cover slip for a more even spread of the stain and sample. Immediately place covered slide in incubator for three (3) minutes at 55°C / 131F to kill sperm. Alternatively, the prepared slide can be held over the ethanol flame (or cigarette lighter) for three (3) to five (5) seconds to kill the sperm cells (flame should just not touch the back of the slide). Wipe the back of the slide when flame is used to

kill the sperm cells or take slide from incubator. Start assessment immediately after.

Assess the sperm cells according to the WHO guidelines (see references) or locally accepted guidelines. Best staining results will be obtained after prepared slide has been left at room temperature for a few hours and assessment can even be performed after 24 to 48 hours.

Results / Outcomes: sperm along peripheral edges of preparation usually stain extremely well in different hues of blue. However, almost no sperm are initially stained in center of preparation, but after a few hours almost all sperm will be stained. Please note that there are a sufficient number of sperm stained for both CASA and manual analysis within 3-5 minutes after preparing slide. These sperm are located more peripherally than in the centre.

Precautions and potential problems: if more than 1.5 µl of semen is used, there will initially be more unstained sperm. Factors that may predispose good staining are accordingly a too high volume of semen as well as highly viscous semen.

References:

1. WHO laboratory manual for the examination and processing of human semen – 5th ed. Switzerland: WHO Press; 2010.
2. Biotech Histochem – van der Horst - 84(6)-299_308-2009-12 – SpermBlue®: A new universal stain for human and animal sperm which is also amenable to automated sperm morphology analysis.
3. Human Reprod - Maree - 25(6)-1369_82-2010_5 – Morphometric dimensions of the human sperm head depend on the staining method used.

Definition of used symbols:

Use before 	Non sterile 	Temperature limit
Keep dry 	Manufacturer 	For single use only
For professional use only 	Read instructions before use 	Do not use when damaged
Production batch 	Number of tests 	Manufacturing date
Certificate of analysis per batch available on request 	Keep away from sunlight 	In vitro diagnostic device