DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Liscia & De Marchi sperm morphology technical information
Technical card code 13-103
Product code 13-103
Pack 3x100 ml or on request
Stability of product properly conserved at 15-25°C 24 months
CND code W01030799

Made in Italy by
DDKItalia S.r.l
Via Marche, 19 ● 27029 Vigevano (I)
info@ddkitalia.com●www.ddkitalia.com

in case of emergency UE number	☎	112
in case of emergency UK number	☎	999
en cas d'urgence Suisse	☎	145

Liscia & De Marchi stain gives good staining of spermatozoa and other cells. It stains the acrosomal and post-acrosomal regions of the head, excess residual cytoplasm, the mid piece and the principal piece. The modified staining technique described here has proved useful in the analysis of sperm morphology and in the examination of immature germ cells and non-sperm cells. Slides stained using Liscia & De Marchi procedure can be permanently mounted and stored for future use in internal quality control programmes. If stored in the dark, they should be stable for months or years.

Method

1) Fixing the air-dried semen smear.
Immerse slides in ethanol 80%, 50% for 30 second.
2) Purified water 30 second.
3) Stain slides into solution one from 30 to 60 second gently agitates.
4) Purified water to remove excess 30 second. Acidic ethanol 4–6 dips of one second. Tap water 5 minutes.
5) Blue water 20 - 30". If manually method is used, keep jar on the top of a white paper to see the virages.
6) Water taps 5 - 6 deep of one second each.
7) Alcohol 96°C 8 - 10 immersion 1 second each.
8) Counterstain with solution 2 for 30 to 60 second gently agitates
9) Alcohol 96°C deep rapidly 10 - 15 times slides for one-second.
10) Alcohol absolute 10 - 15 deep of one second each.
11) Xylen 10 deep of one second.
12) Mount with DdMount

Note 1. Ethanol fixation causes dehydration of the cells. Therefore, smears taken directly from the fixation step in 95% ethanol to staining may need only 10 seconds in the 80% ethanol, whereas smears that have air-dried after fixation must remain longer (2–3 minutes) in the 50% ethanol.
Note 2. In Step 6 above, start with 4 dips and continue until results are satisfactory. This is a critical step, as the duration of destaining dramatically alters the final stain intensity. If this step is omitted, spermatozoa and background will be dark. Increasing the number of dips will make spermatozoa and background fainter.

Note 3. The slides can be viewed unmounted or mounted.

Examining the stained preparation

With stained preparations, a ×100 oil-immersion bright field objective and at least a ×10 eyepiece should be used. Clearer images are obtained when a fluid of similar refractive index (RI) to those of cells (approximately 1.5) and glass (1.50–1.58) is placed between the lens and the unmounted section or glass coverslip. This is usually immersion oil (RI 1.52). Mounting media have similar refractive indices (1.50–1.55).

Mounting media
Slides can be viewed un-mounted or mounted (without or with a cover slip attached). Mounting the slides permits long-term storage, so that they can be reassessed if necessary and used in an internal quality control programme. The refractive index (RI) of mountants after drying (1.50–1.55) is similar to that of glass (1.50–1.58), and the best optical quality comes with the use of immersion oil with a similar RI (1.52).

DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Liscia & De Marchi sperm morphology technical's information's
Technical's card 13-103
Product code 13-103

Result

The head is stained pale blue in the acrosomal region and dark blue in the post-acrosomal region. The midpiece may show some red staining and the tail is stained blue or reddish. Excess residual cytoplasm, usually located behind the head and around the midpiece, is stained pink or red

Classification of normal sperm morphology

Assessment of sperm morphology is associated with a number of difficulties related to lack of objectivity, variation in interpretation or poor performance in external quality-control assessments. The method recommended here is a simple normal-abnormal classification, with optional tallying of the location of abnormalities in abnormal spermatozoa.

References

WHO laboratory manual for the examination and processing of human semen, 5th edition, WHO, 2010

Endnotes

1 The timing suggested in the leaflet are approximate and may vary according to your specific needs. If they are used intensively, for staining solutions may lose their dyes, so it is necessary to extend the time of staining solutions, or replace with new products.
2. Include positive control slides in each session.
3. Some hydraulic systems deliver acidic water, unsuitable for use for the part of the procedure for the blue coloration. If tap water is acidic, instead using a dilute alkaline solution, for example, water buffered by Scott.
4. The presence of purple or red-brown nuclei a blue color indicates unsatisfactory.
5. If you over-eosin staining, nuclear staining may be masked. If done correctly, with eosin staining shows a three-tone effect. To increase the differentiation of eosin, extend the time of immersion in alcohol, or use a first alcohol with a higher water content. You can adjust the times of immersion in alcohol to obtain an adequate eosin staining.
6. We do not recommend the addition of stock solution in the working solutions of haematoxylin and eosin.
7. Avoid excessive drag (carryover) of water solutions in alcoholic eosin.
8. The data generated by this procedure are to be used only to support the diagnosis and should be evaluated in conjunction with other tests and diagnostic data