LABQUALITY

External Quality Assessment Scheme

Histological staining techniques 1, 2018: Iron, reticulin

Thank you for participating in this round of histological staining techniques.

SPECIMENS

The quality of iron and reticulin stainings were evaluated in this round. Each participant received two slides with unstained sections, one for iron stain and one for reticulin stain.

Reticulin staining was performed on a tissue section from follicular lymphoma and iron staining on a tissue section from an autopsy specimen of adrenal gland with hemochromatosis. The sections were cut from paraffin embedded tissue fixed in 10% neutral phosphate-buffered formalin. The sections were taken on pure X-tra™ Adhesive Precleaned Micro Slides (Leica) and attached overnight at +40 ºC followed by 30 min at +60 ºC temperature.

63 laboratories from 11 countries participated in this round. Following number of stained slides was returned: iron 54 slides and reticulin 52 slides. Two laboratories did not return their slides at all.

RESULTS

A numerical score given in a six-step scale 0–5 is based on consensus. The results of all participants are presented in a table form. A laboratory specific score report is enclosed as well. Scoring reports help your laboratory in following the development of your own stainings. Guidelines how to interpret the reports can be found under ”LabScala user instructions” in LabScala. The scoring principles are presented below.

SCORING

Evaluation scale: 0-5

3-5 points indicate good enough staining for diagnosis. 0-2 points mean that the staining is uncertain for diagnosis or failed.

5 points = optimal = excellent
4 points = almost optimal = practically faultless, slight over/understaining, slightly uneven or patchy staining
3 points = good enough for diagnosis, but distinct over/understaining, uneven or patchy staining, stain deposits etc.
2 points = borderline = weak staining, uncertain for diagnosis
1 point = poor = failed, some scanty but inadequate staining observed/ notifiable overstaining
0 point = negative staining - failed

Scores	Iron (n)	Reticulin (n)
5	35	6
4	13	18
3	5	17
2	-	9
1	-	1
0	1	1
Mean	4.5±0.9	3.3±1.1

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Final report

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Items sent	2018-04-03
Round closed	2018-05-08
Report released	2018-07-09

The report contains
- The results of all participants
- Laboratory specific scores

Request for corrections

Inquiries about this round, including questions of possible errors in result processing, should be at Labquality's office within a month from the date of this letter.

Expert of the round

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Evaluation of the slides with experts

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Iron

Endogenic iron is found in hemoglobin, myoglobin and certain enzymes like cytochrome oxidase and peroxidases. Iron is mainly stored in the bone marrow, and when released from degrading cells it is effectively circulated in the body. Lack of iron is usually due to hemorrhage. Excess iron is stored in the body e.g. following blood transfusions or drugs for iron deficiency. Iron accumulates as hemosiderin mostly in the bone marrow, spleen and liver. Hemochromatosis is a rare hereditary condition where the mechanism controlling iron absorption is disturbed leading to accumulation of iron to various organs. The first specimen to be stained in this round was from the adrenal gland of a hemochromatosis patient.

The most used method for staining iron is to demonstrate ferric iron with ferrocyanide (eg. Perls' or Lillie's methods). The end result is a blue ferriferrocyanide precipitate (Prussian blue).

Criteria of the iron stain
- The end result should be a sharp blue precipitate well distinguished from the background
- No over/understaining
- The staining should be even and clean (no excess stain or stain deposits, no contamination)

Results
All (53) laboratories succeeded in getting an acceptable result of the iron stain. Only one laboratory was given 0 points because they had stained the slide meant for the reticulin staining. 35 laboratories (64.8%) received 5 points, 13 laboratories (24.7%) got 4 points and 5 laboratories (9.3%) 3 points.

Protocol information
The protocol information sheet was filled and returned by 40/54 laboratories. Concerning many laboratories the information was unfortunately scarce. According to the given information iron was stained with the potassium ferrocyanide method by most (16) laboratories. The most popular counterstain was kernechtrot (11 laboratories). Nuclear red as counterstain was used by 8 laboratories and other counterstains included eosin (2), neutral red (2), Mayer's hematoxylin (1), Mayer's carmalum (1), pikrosaturn red (1).

Manual and automated stainings
Most laboratories (32) stained iron manually. Eight laboratories used a staining instrument.

Staining instruments and kits
The following staining instruments were used: Dako Artisan (4 laboratories), Ventana Special Stainer (3) and Sakura Prisma (1). Staining kits were also used: Dako Artisan Iron Staining Kit (4 laboratories), Ventana Iron Special Stain Kit (3), Bio-Optica Iron Stain kit (2), Biognost-Hemognost Perls Kit (1) and Diapath Perls Staining Kit (1).

Problems in the iron stain
The most common defect in the iron stain was slight overstaining leading to faint background, and small impurities on slide. These defects cost one point. Slides worth 3 points showed more overstaining and background or stain deposits. One slide was understained, weakly positive, but still diagnostic.

Reticulin

Reticulin fibers can be stained with common tissue dyes or with the metal impregnation method. The latter is more sensitive and mostly silver, actually its salt silver nitrate ($AgNO_3$) is used as an alkaline solution. (eg. Gomori's, Gordon & Sweets' methods). Reticulin fibers have low affinity to the silver solution and the staining requires heavy pretreatments in solutions like potassium permanganate, oxalic acid and ammonium ferric sulphate.

Reticulin staining is used e.g. in lymphoma diagnostics and the second specimen to be stained in this round was from follicular lymphoma.

Criteria of the reticulin stain
- Silver should be reduced black in fibers of all sizes as the end result and also in very thin reticular fibers
- Positivity should be well distinguished from the background

- Silver should not be reduced to cells or ground substance
- The staining should be even and clean (no excess stain or stain deposits, no contamination)

Results
Most laboratories stained reticulin as a silver stain, and there were problems. Only 6 laboratories (11.5%) got 5 points. Four points were achieved by 18 laboratories (34.6%) and 3 points by 17 laboratories (32.7%). Non-acceptable stainings were performed by 11 laboratories (21.2%), 9 of which received 2 points, one got 1 point and one was given 0 points (wrong slide stained).

Protocol information
The protocol information sheet was filled and returned by 38/52 laboratories. Protocol information was quite inadequate for many laboratories. The information available showed that laboratories stained reticulin with very many modifications. Even the laboratories whose staining was worth 5 points used varying methods. Three 5 point laboratories used a quite similar protocol following the order: potassium permanganate – oxalic acid – ferric ammonium sulphate – silver solution – formalin – gold chloride – sodium thiosulphate – nuclear stain (nuclear red/light green/kernechtrot). One 5 point laboratory stained with Ventana's special stainer.

Manual and automated stainings
Most laboratories (29) stained reticulin manually. Nine laboratories used a staining instrument.

Staining instruments and kits
The following staining instruments were used: Ventana Special Stainer (5 laboratories) and Dako Artisan (4). Staining kits were used: Bio-Optica Silver Impregnation Kit (6 laboratories), Ventana Reticulin Special Stain Kit (5) and Dako Artisan Reticulin Staining Kit (4).

Problems in the reticulin stain
It is challenging to get a perfect result from silver stains. All reaction vials have to be ultraclean and no metal equipment should be used. Silver tends to be reduced by metal and all kinds of impurities. Controlling the silver reaction is important. Concentrations, reaction times and temperatures should be tested to be optimal. Losing one point was possible for several reasons: slightly uneven staining or impurities, faint background, slightly weak silver reaction, slightly too heavy counterstain. Two points were lost for the same reasons, but the defects were considerably larger. In many of the non-acceptable stainings, the counterstain was too dark thus totally overpowering the positivity or the silver reaction was not working and the reticular fibers remained unstained. One point was given to one slide that was completely black. And one laboratory got zero points for staining the slide meant for the iron stain.

CONCLUSIONS

The laboratories performed the iron stain very well. Some stains could be improved by adjusting the staining conditions to eliminate background. Reticulin stain was problematic for a number of laboratories. Maybe the simplest advice is to ask for a protocol of a stain worth 5 points from Labquality.

End of report