Red Blood Cell removal from Heavily Bloodstained Diagnostic Cytology Serous Fluid Samples using Sigma Red Blood Cell Lysing Buffer

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INTRODUCTION

When serous samples such as pericardial, peritoneal or pleural fluids arrive heavily contaminated by blood it can be impossible to visualise the cells of importance and therefore without red blood cell removal may cause poorly cellular preparations, likely to be reported as false negative if not re-processed for blood removal. Blood removal from heavily bloodstained serous fluid samples and sample deposits in this laboratory had been historically undertaken using Cytolyt solution. Cytolyt, valid only for pre-treatment to follow in PreservCyt solution for LBC ThinPrep Papanicolaou (PAP) preparation, visibly appeared to not facilitate air dried preparations as made in this laboratory. (May-Grunwald Giemsa (MGG) preparations were noted by the author to be particularly poor when using Cytolyt). Also this had not previously been validated for intended use by the previous Non-gynae cytology staff.

It is best practice to make an air-dried slide for MGG staining and a wet fixed slide preparation for PAP staining for serous fluid cytology diagnosis (1).

A simple method of red blood cell removal was required by our laboratory in order that all other cells in the sample remain intact, allowing clarity for visualising diagnostic material, fixed and stained appropriately for MGG and PAP staining prior to assessment, whilst being amenable to cell block Immunohistochemistry.

METHOD

Unused samples and sample deposits are kept refrigerated for 7 days and then checked that they are reported final plus 48 hrs before disposal. Any unused heavily bloodstained samples ready for disposal were used to test the recommended Sigma Red Blood Cell Lysing Buffer (2) for blood removal. As there was no Standard Operating Procedure offered by NEQAS CPT technical update meeting on or after November 2021 and this department already used Versalyse for Flow Cytometry on Diagnostic cytology samples, the IFU for Versalyse was used as a basis for a trial method for the Sigma Lysing Buffer(3).

Deposits were split between; No pre-treatment, current CytoLyt method, Versalyse method and Sigma Lysing Buffer (using the Versalyse IFU). Cytofoam cell blocks were made from the remaining deposit for CytoLyt and Sigma Lysing Buffer to compare with natural clots found in the original samples in order to validate the Immunohistochemistry.

We decided this method should NOT be carried out on samples with a single universal.

- <25ml of heavily bloodstained fluids are processed for direct slides made from the buffy layer and left for subsequent cell foam blocks if required.
- Any clots should be removed and processed for histology in the normal manner.
- >50ml - Universals 1 & 2 should be processed for Sigma Lysing solution (Ammonium chloride – below). Tube 3 should be saved as original, untreated sample (with clots taken out).

1. Following centrifugation of the Falcon tube (up to 50ml) at 3000 rpm for 5 minutes the supernatant should be pipetted off into the original empty universals marked “S” leaving a bloodstained cell deposit.
2. Direct PAP and direct MGG slides are made from this original deposit buffy layer and slides marked as “Untreated”
3. Up to a maximum of 15ml of the Sigma Ammonium chloride lysing solution is added to the cell deposit and mixed by inversion for 1 minute and left to rest for 20 mins minimum whilst preparing other case samples.
4. Centrifuged to a cell deposit, the supernatant is decanted into waste and the deposit reconstituted with a minimum of 15 ml with HANKS solution, mixed and centrifuged as a cell wash.
5. After centrifugation the supernatant can again go into waste. The deposit remaining should be mixed and used for either direct or cytospin preparations.
6. PAP and MGG slides should be marked Rpt + Amm.chloride.
7. The labelled untreated and treated cytology slides and any histology clots are assigned to the Pathologist for reporting.
8. All reagent consumable stickers are attached to the back of form and histology H&E request slip.

CONCLUSION

The aim of this trial using Versalyse and Sigma RBC lysing buffers was to find a method that would allow samples that are heavily bloodstained give excellent morphological preparations for MGG air-dried staining as well as the PAP fixed preparations.

Both Versalyse or Sigma RBC lysing buffer could be used but in order to keep solutions in each department separated for stock control and the prices being similar we validated the IHC for Sigma RBC Lysing buffer and are using the Sigma RBC Lysing buffer in future.

REFERENCES

1. Minimum recommendations for sample preparation and staining Serous fluids, RCPath Tissue Pathway for Diagnostic Cytopathology, October 2019.
2. UK NEQAS CPT Diagnostic Cytology refresher course 23.11.21 Webinar, Synlab, Taunton.
3. Versalyse ™ Lysing Solution, https://www.bc-cytometry.com, 14.03.23.

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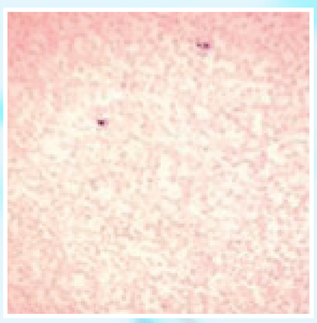
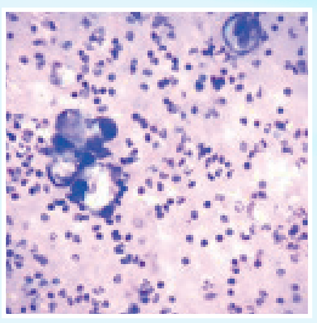
RESULTS

The cytology slides prepared for MGG staining were much improved in both the Versalyse and Sigma Lysing Buffer treated samples to that of CytoLyt or untreated, whilst excellent staining for PAP remained. The benefits are seen on samples containing scanty malignant cells (Fig 1a and b)

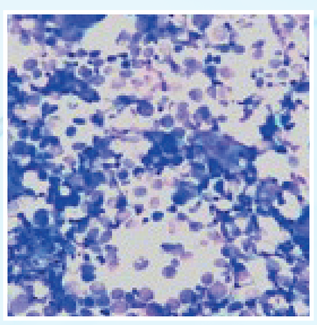


1a) Direct spread before treatment (MGG X 100)

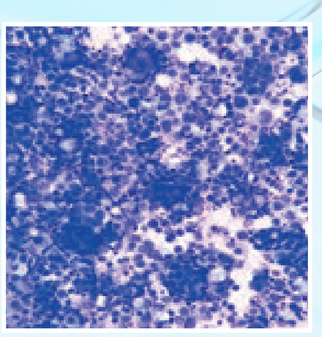


1b) Cytospin MGG after treatment with Sigma Lysing buffer (x100)

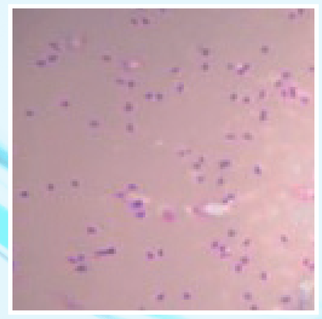
The lymphocytic fluids were greatly improved in lymphocyte morphology (Fig 2a and b and 3a and b)



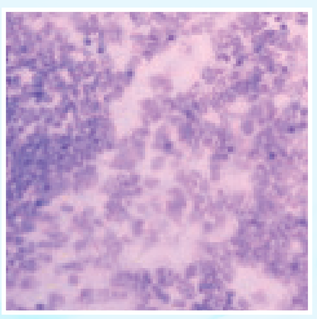
2a) Pleural fluid pre-treated with Cytolyt (x100)



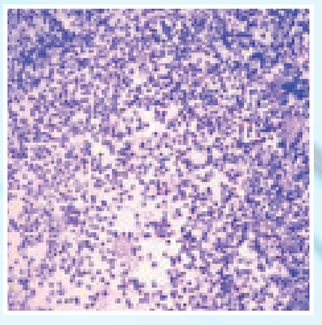
2b) Same pleural fluid pre-treated with Lysing buffer (x100)



3a) Untreated sample MGG X 100



3b) CytoLyt treated sample MGG X 100



3c) Sigma RBC lysing buffer MGG X 100

RESULTS CONTINUED

The Cytofoam™ cell blocks made from remaining deposit for both CytoLyt and Sigma Lysing Buffer were validated against natural sample clots with a panel of markers – all results appeared in concordance.