Evaluation of an agar cell block method to improve cell yield in non-gynaecological cytology specimens.

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Objective

To determine if modifications to our agar cell block (CB) method improved cell yield for fine needle aspiration (FNA) and for body fluid specimens.

Methods

We recently modified our agar CB method (agar method) to incorporate two steps used by Varsegi and Shidham[1] in a method described for making CBs from ThinPrep specimens. Both methods involved concentration of cells by centrifugation, suspension of the cell pellet in liquified agar followed by further centrifugation. The resulting agar pellet is cooled to solidify prior to embedding for processing and microtomy. The modifications included the use of a small flat bottom 5mL tube for the final centrifugation step and a visual marker to indicate the correct level for sectioning. The latter involves the addition of a 2x2mm (approx) piece of banana skin that is spun with the cell pellet and comes to lie with the cells on the bottom of the flat bottomed tube. These modifications are designed to concentrate the cells on a single plane and provide a visual marker to indicate the plane for optimal sectioning.

After an initial trial period the new method (marker method) was used prospectively for 210 consecutive FNA specimens (fluid collected or washed out of the needle) from a wide range of sites and 135 fluid specimens (serous effusions and peritoneal washings). H&E stained CB slides were evaluated to determine if sufficient material was present for diagnostic interpretation and /or ICC. Results were compared with historical data for 250 consecutive specimens (158 FNAs and 92 fluids) prepared using our old (agar) method.

Results

The marker method was simple to perform and resulted in a higher proportion of adequate CBs. Adequacy rates for the agar Vs marker method were: total FNA 35% Vs 51%; FNA non-attended 32% Vs 48%; attended FNA 41% Vs 59%; fluids 79% Vs 86.5%. The improvement was statistically significant for all FNAs (P=0.003), non-attended FNAs (P=0.017) and for FNAs with a scientist in attendance (P=0.05), but not for fluid specimens (P=0.2).

Conclusions

The modifications significantly improved the proportion of CB preparations with adequate cells, especially for poorly cellular samples, such as most FNA washouts. The visual marker ensures specimens are not over- or undercut and examination of the H&E slide quickly allows assessment of whether the correct level has been sampled. This is important as adequate CBs often allow greater precision of cytological diagnosis through the application of ancillary tests.

1. Varsegi GM Shidham V. Cell block preparation from cytology specimen with predominance of individually scattered cells. http://www.jove.com/details.stp?id=1316 doi: 10.3791/1316 J. Vis Exp. 29 (2009).

Gastrointestinal Stromal Tumours

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Clinical Presentation

Patient 1: A 56 year old male had an incidental finding of a 2 cm hypo-echoic submucosal lesion in the gastric body upon investigation of iron deficiency.
Patient 2: A 73 year old female presented with a 12 cm diameter gastric lesion.
Patient 3: An 87 year old female presented with a 12-15 cm hypo-echoic peritoneal mass.

Cytological findings

Patients 1 and 3 had similar tumour morphology comprising cells with a spindle cell appearance. Main features include: dense cohesive groups suggestive of fascicular arrangement; loosely fibrillary stroma; nuclear 'streaming'; elongated 'cigar' shaped nuclei; coarsely granular and evenly distributed chromatin; small nucleoli; low nuclear-cytoplasmic (N:C) ratio and moderate amounts of delicate cytoplasm.

Patient 1 also had areas of epithelioid morphology. This comprised clustered groups of round cells with a low N:C ratio and central uniform round to oval nuclei. Chromatin was coarsely granular and evenly distributed; nucleoli were small.

Follow-up studies

Histological features of both spindle cell and epithelioid variants recapitulate what was found in cytology. The resected tumour in Patient 2 was found to be within the muscularis propria.

All specimens were positive for C-kit mutations using immunocytochemistry (ICC).

Discussion

There is excellent morphological correlation between cytology, histology and cell block preparations in all three patients. ICC is important in identifying the origin of gastrointestinal tumours and for dictating patient management. C-kit is a highly specific ICC marker for GIST's and is essential in differentiating GIST's from other lesions such as leiomyoma, a benign tumour.

C-kit positive tumours can be treated with targeted Imatinib Mesylate therapy and surgical resection.

1. Avery A, Faigel D, Heinrich MC, McGreevey LS, Rader AE, Wait CL: Fine-needle aspiration biopsy diagnosis of gastrointestinal stromal tumors using morphology, immunocytochemistry, and mutational analysis of c-kit. Cancer Cytopathology 2001;93,4:269-275.
2. Ghafari S, Gu M, Lin F, Nguyen PT: Cytologic Diagnosis of gastrointestinal stromal tumors of the stomach by endoscopc ultrasound-guided fine-needle aspiration biopsy: Cytomorphologic and immunohistochemical study of 12 cases. Diagnostic Cytopathology 2001;25,6:343-350.