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CONCOURS EXTERNE

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BAP A

Emploi-type : « Assistant·e ingénieur·e en expérimentation et instrumentation biologiques »

SESSION 2023

Épreuve écrite d'admissibilité

Durée : 3 heures

Coefficient : 4

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1- Que signifient les acronymes suivants :

EPST :

UMR :

BAP :

IRM :

AAV :

OGM :

2- Quel volume faut-il pipeter d'une solution mère d'acide chlorhydrique 36% pour faire 400 ml d'une solution 0.6M final (d=1,2 PM=36) ?

3- Quelle est la définition du pH ? Quel est le pH d'une solution à 10^{-2} M d'acide chlorhydrique?

4- Vous souhaitez faire une gamme étalon à l'aide de la BSA (solution mère à 2 mg/mL), afin de déterminer la concentration protéique de votre échantillon.

Remplissez ce tableau de volumes à pipeter :

Quantité de BSA (µg):	0	25	50	100	200
Volume de BSA (µl)					
Réactif de BRADFORD 5X (µl)					
Eau distillée (µl)					
Volume final (µl)	200	200	200	200	200

5- Vous disposez d'une protéine protB, d'un anticorps monoclonal de souris anti-protB, d'une anti-immunoglobuline de souris couplée à la péroxydase. On vous demande de doser la protéine protB par la technique d'ELISA.

a) Définir le sigle « ELISA » :

b) Décrire le protocole à mettre en place :

-

-

-

-

-

-

-

6- Compléter les phrases suivantes :

a) Afin de visualiser un ADN de 100 paires de bases, la migration peut se faire à l'aide d'un gel

_____

b) Lors d'une électrophorèse SDS-PAGE, les protéines migrent en fonction de leur(s)

_____

7- Quel est le rôle du SDS dans une électrophorèse SDS-PAGE ? A quoi peut servir le β-mercaptoéthanol ou DTT dans cette technique ?

8- Relier par des flèches les types de chromatographie correspondant aux résines présentées à gauche :

Mono-S	•	
Nickel – agarose	•	• Affinité
Héparine – agarose	•	
DEAE	•	• Echange d'ion
G50	•	
Protéine G- sépharose	•	• Gel filtration

9- Après la purification d'un ADN plasmidique, que mesure la densité optique à 260nm et à 280nm ? A quoi sert le rapport DO 260/280?

10- Comment appelle-t-on le fait d'introduire un ADN plasmidique dans une cellule procaryote ? Par quelles techniques peut-on y parvenir ?

11- Qu'est-ce qu'un clonage ? Citez un exemple et son utilisation.

12- Qu'est-ce qu'une enzyme de restriction ?

13- Quelles sont les différences entre un organisme procaryote et un organisme eucaryote ? Citer trois différences

-

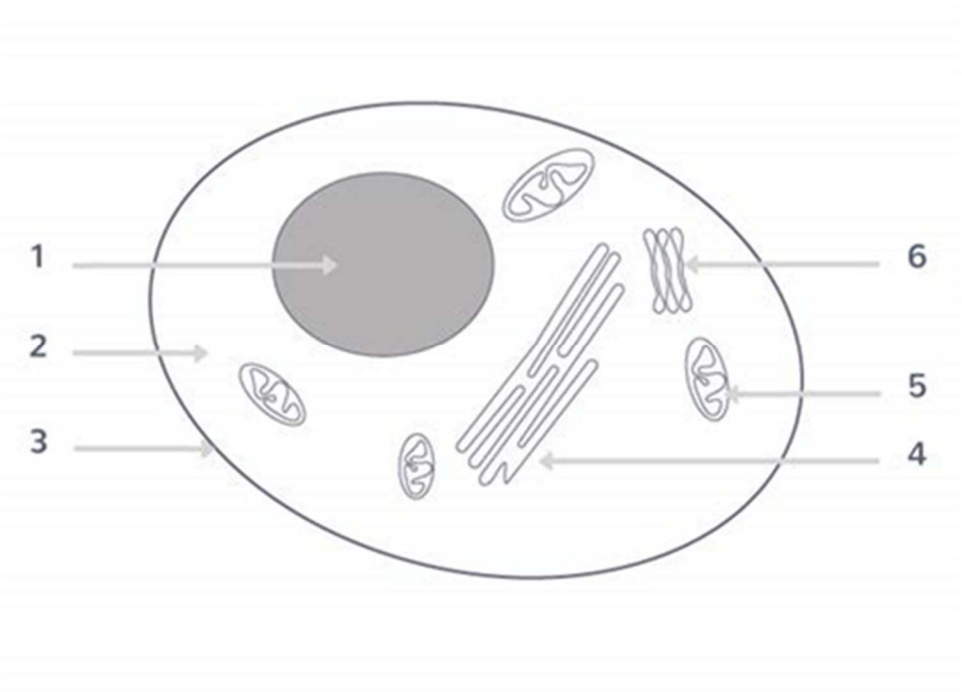
-

-

14- Vous devez congeler une lignée cellulaire dans du DMSO. Qu'est-ce que le DMSO et à quelle concentration devez-vous l'utiliser ?

15- A quoi sert la trypsine en culture cellulaire ?

16 -Légender le schéma ci-dessous :



1 :

2 :

3 :

4 :

5 :

6 :

17- Dans quel(s) environnement(s) devez-vous manipuler une lignée cellulaire humaine potentiellement pathogène ?

18-Qu'est-ce qu'un CMR ? Donnez un exemple, et les EPI nécessaires à sa manipulation.

19- Quels sont les dangers associés aux pictogrammes suivants :

_____

_____

_____

_____

_____

_____

20- Vous travaillez dans une équipe qui mesure le dihydrogène (H_2) produit par le microbiote intestinal dans l'air expiré de patients. Des valeurs inférieures à 25 ppm (parties par million) sont considérées comme normales. Une intolérance alimentaire provoque une forte hausse de l'hydrogène exhalé. Vous avez à votre disposition un analyseur de gaz par chromatographie (un Quintron®) qui est déjà calibré. Le Quintron® permet l'analyse d'un échantillon de 20 mL de gaz expiré et sa gamme de mesure est comprise entre 0 et 500 ppm.

Analyseur de H_2 expiré Quintron® et son système de prélèvement

Analyseur de H_2
ASI

Sac étanche au gaz

Vous recevez un nouvel appareil portable ASI, muni d'une pompe réglable entre 200 mL/min et 2L/min, qui analyse en continu la valeur de H_2 grâce à un capteur spécifique. La gamme de mesure est la même que celle du Quintron®. Vous avez à votre disposition tout le matériel courant d'un laboratoire de biologie (tuyaux de différents diamètres, pipettes, vannes 3 voies, seringues de différents volumes, …) ainsi que du matériel spécifique :

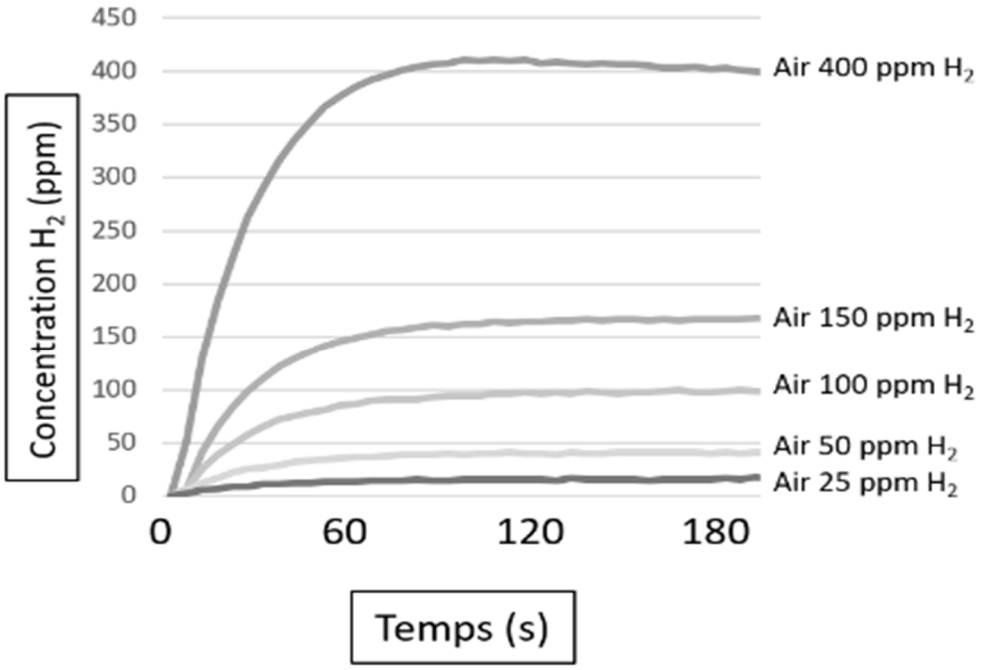
- **des sacs de 3L, étanches au gaz, munis d'une vanne et d'un embout,**
- **Un compresseur à air comprimé**
- **Une bouteille B50 contenant 97% de N_2 et 3% de H_2.**

a) Vous voulez vérifier le bon fonctionnement de la pompe du capteur ASI : imaginez une expérimentation qui permette d'estimer le débit de la pompe.

b) Quelle est la concentration de H2 dans la bouteille B50 en ppm ?

c) Quelle dilution faut-il préparer dans le sac pour être environ au milieu de la gamme de mesure des 2 appareils ?

d) La mesure de 20 mL de ce mélange avec le Quintron vous donne 292 ppm. Vous calibrez l'appareil ASI sur 292 ppm en le branchant au sac de référence puis vous préparez une gamme de mesures et obtenez les résultats suivants :



Que peut-on dire du temps de réponse de l'appareil ?

-

e) Comment garantir la validité de la mesure dans l'air expiré d 'un patient ?

21- Le protocole ci-dessus détaille l'expression, la purification et la caractérisation d'une protéine recombinante (GST-OSMR FN III). A l'aide de ce dernier, répondez aux questions ci-après :

Expression and purification of GST-OSMR FNIII domains from LB medium

A single colony was picked up from the LB agar plate using a micropipette tip and dipped in 2 ml of LB medium containing ampicillin(100 ug/ml). The culture was incubated at 37 °C for 10–12 h in an orbital shaker at 180 rpm. From this culture, 50 µl of inoculum was transferred into 500ml of LB medium containing ampicillin and grown at 37 °C till the bacterial cell density reached an absorbance of 0.6 at OD_{600}. When the required OD was observed, cells were induced with IPTG (1mM) and incubated for another 3 h. Cells were then pelleted down at 4000 rpm, 4 °C, for 15 min. Cell pellets were dissolved in a lysis buffer containing a protease inhibitor cocktail. The cell suspension was sonicated, and the lysate obtained was spun down at 4000 rpm for 15 min at 4 °C. The supernatant obtained was transferred into fresh 15ml centrifuge tubes containing 300 µl of glutathione-agarose beads, rotated at 4 °C for 2 h, and then spun down at 3000 rpm for 5 min at 4 °C. The supernatant was discarded, and the beads were washed thoroughly to get rid of unspecific contaminating proteins. GST-tagged fibronectin domains bound to the beads were eluted by adding 2 ml of elution buffer, mixed thoroughly, and then centrifuged at 3000 rpm, 4 °C, for 5 min(this step was repeated three times to remove the bound protein domains completely). Finally, eluents obtained for each domain were pooled up separately in 15ml centrifuge tubes. Then the eluents were dialyzed (10 KDa cutoff dialysis bag) overnight in a dialysis buffer and concentrated by centrifugation in spin columns at 3000 rpm, 4 °C, for 20 min.

Immunoblotting

Purified fibronectin domains were loaded onto 10% SDS-PAGE and were initially run at 80 V until they crossed the stacking gel, then at a constant voltage of 100 V for approximately one and a half hours. After the completion of SDS-PAGE, western transfer was performed at 100 V for approximately 90 min. After confirming the protein transfer with the ponceau stain, the membrane was blocked for 1 h with 5% skimmed milk prepared in 1X TBST. After blocking, the primary antibody(anti-GST antibody) prepared in 5% skimmed milk(1:1000 dilution) was added to the membrane and incubated for 2 h at room temperature. Later, the membrane was washed thrice with TBST(1X) and incubated with anti-rabbit antibody (secondary antibody) prepared in 5% skimmed milk for 1 h at room temperature. Finally, after the completion of secondary antibody incubation, the membrane was washed thrice with TBST(1X) and processed with enhanced chemiluminescence reagent(Amersham) for observing protein bands.

a) Expliquer brièvement l'intérêt d'ajouter de l'ampicilline dans le milieu de culture.

b) Pourquoi suivre l'absorbance à 600 nm ? Quel équipement utiliseriez-vous ?

c) Quelle est la fonction de l'IPTG ? Expliquer brièvement le principe de cette étape.

d) Pourquoi un western blot est réalisé après le SDS-PAGE ?

e) Après le western blot, il est mentionné que "the membrane was blocked for 1h with 5% skimmed milk". Quel est l'intérêt de cette étape ?

f) Traduire les termes suivants :

- Pellets :

- Buffer :

- Discarded :

- Cutoff :

- Room temperature :

g) Vous avez à votre disposition du TBST 10X, du lait écrémé en poudre et 1 tube de l'anticorps primaire anti GST. Détailler la marche à suivre pour préparer 20 mL de solution d'anticorps comme demandé dans le protocole.

La figure ci-dessous représente les résultats du SDS PAGE (A) et Western Blot (B) de l'expression/purification de différentes formes de la protéine :

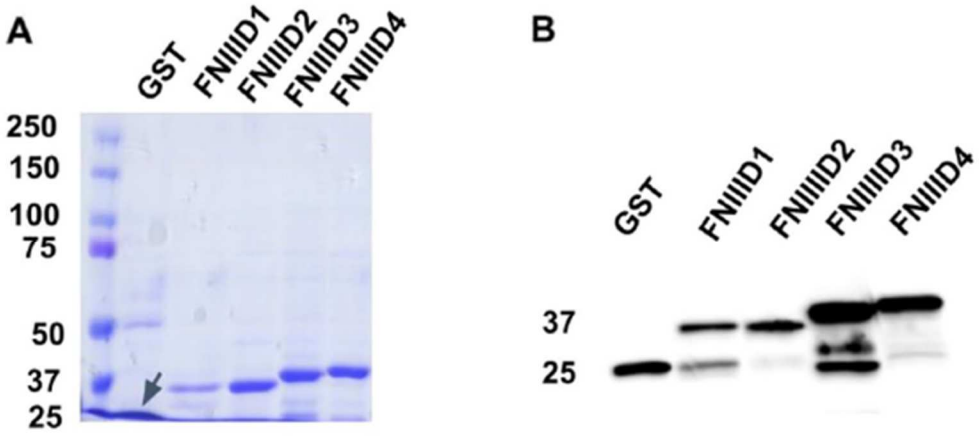


Fig. 4 SDS PAGE of Purified FNIII domains and Immunoblotting: **A** Purified and concentrated GST-tagged FNIII domains were loaded on 10% SDS PAGE. Lane1: Protein Marker, Lane2:GST alone, Lane3:GST-FNIIID1, Lane4:GST-FNIIID2, Lane5: GST- FNIIID3, Lane 6:GST-FNIIID4. **B** The purity of the protein domains was confirmed with Immunoblotting using an anti-GST antibody

h) Quel est le poids moléculaire du Tag-GST ? En quelle unité est-il exprimé ?

i) Les protéines purifiées et caractérisées sur le gel d'électrophorèse se situent toutes sur la partie inférieure du gel. Qu'auriez-vous pu changer dans le protocole du SDS PAGE pour obtenir une meilleure séparation ?

j) Sur la piste 2 (FNIIID1) du western blot (B), à quoi correspondent les deux bandes visibles ?

k) Expliquer le résultat de la piste 4 (FNIIID3) du western blot (B) :

22- <u>Lire l'article (joint avec le sujet)</u> :

« Sponsored and reviewed by ICCS Quality and Standards Committee Title: Tissue Disaggregation Methods for Flow Cytometric Immunophenotyping »

Written by: Bethany Vallangeon, MD and Ameet R Kini, MD, PhD ; Date: 8/6/2019.

<u>Répondre aux questions suivantes (en français) :</u>

a) Dans le cadre d'une analyse en cytométrie en flux (CMF) quelle est la caractéristique de la suspension à obtenir ?

b) Quelles sont les deux qualités à préserver pour les cellules ainsi obtenues ?

c) Quel est le but de ce travail ?

d) Pour quelle raison l'obtention d'une suspension cellulaire mono-dispersée, dans le cadre d'une analyse de lymphome, peut-elle être compliquée ?

e) Nommer les 5 méthodes de dissociation étudiées :

f) Quel type de protocole de dissociation est en général utilisé ?

g) Ses principaux avantages ?

h) Quel est le principal avantage de la dissociation par agitation au Vortex ?

i) Citer un désavantage de la méthode de dissociation au Vortex?

j) Trois arguments commerciaux avancés pour choisir la dissociation à la machine ?

k) Pourquoi est-il important de conserver une bonne viabilité cellulaire lors d'un immuno-phénotypage en CMF ?

l) Quels sont les deux fluorochromes fréquemment utilisés pour quantifier la mort cellulaire ? Et leur mode de fixation ?

Sponsored and reviewed by ICCS Quality and Standards Committee

Title: Tissue Disaggregation Methods for Flow Cytometric Immunophenotyping

Written by: Bethany Vallangeon, MD and Ameet R Kini, MD, PhD

Date: 8/6/2019

INTRODUCTION

Successful flow cytometry analysis requires a single-cell suspension; therefore, peripheral blood, bone marrow, and body fluid samples are all very suitable sample types. In contrast, tissue samples such as lymph node and extranodal tissue require processing into single-cell suspension before flow cytometric analysis can be performed. It is advisable that the selected method of tissue processing preserves the cell viability and antigenicity as much as possible. In particular, diagnosis of large cell lymphoma by flow cytometric analysis is often difficult; up to 25% of large cell lymphomas may not be detected.[1]

The tissue disaggregation method used can adversely impact immunophenotyping, including possible cell loss, decreased viability, non-specific antibody binding, and variable alterations of antigenicity. It is critical to select a method that is best for your assay and your laboratory and to validate that protocol in house.

Our goal is to compare and contrast different tissue disaggregation methods, discuss the factors that impact the results, advantages and disadvantages, and provide a starting point for choosing and validating the optimal method for your laboratory.

METHODS

The disaggregation of tissue into a single-cell suspension is a critical step in the flow cytometric analysis of hematologic malignancy. Lymphomas dissociate variably based on the nature of involved tissue (extracellular matrix); as an example, lymphomas involving extranodal sites such as skin may present a technical issue. Historically, various methodologies of mechanical dissociation have been utilized including scraping, mincing, fine needle aspiration and automated techniques.

Mechanical Manual Disaggregation Techniques

1) Needle[2]
2) Mesh[3]
3) Scraping[4-5]
4) Mincing[5]
5) Vortex[6]

In general, manual methods involve using a combination of needle, surgical blade, and/or mesh to disaggregate tissue and washing cells with an appropriate media. The cells can then be collecting utilizing a disposable pipette and transferred into a processing tube. The resulting sample can be washed with media or PBS/azide and the cell button resuspended in PBS/azide or media and subsequently counted to adjust the cell concentration.

Figure 1 (below) illustrates the procedure for tissue disaggregation using the needle method while Figure 2 illustrates the mesh technique.

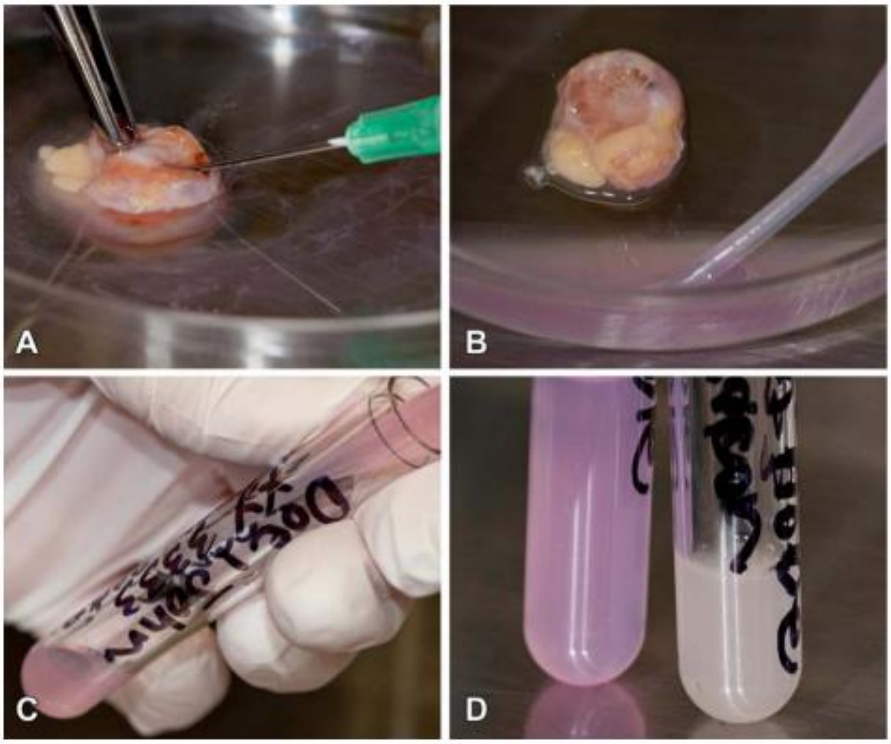


Figure 1 (from Reference 2). Needle Manual Method. A: Puncturing tissue with needle and infusing PBS solution, (B) Aspiration of cell suspension with plastic pipette, (C) Collection tube, and (D) Cell suspension recovery comparison (left tube = manual; right tube = automated).

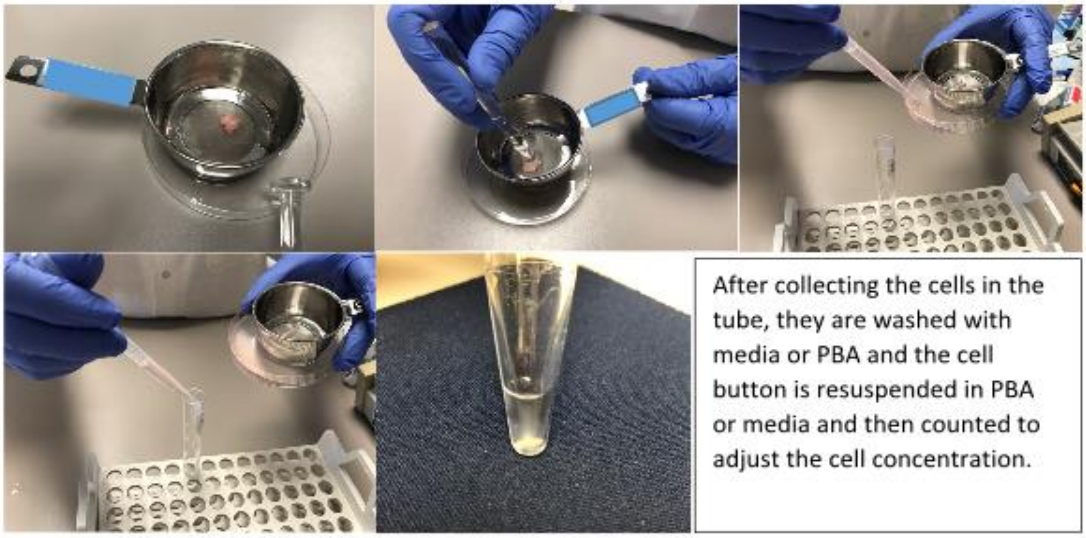


Figure 2 (from Reference 3). Mesh Manual Method. In mechanical disaggregation, the tissue sample is disaggregated by simply placing it on a wire mesh over a Petri dish and gently pressing down on the tissue with a glass pestle (or with tissue scissors) and washing cells with appropriate media. The cells can be collected by using a disposable pipette and transferring the cells into a processing tube.

The most prominent advantage of using a manual method is in its notably gentler technique[2]; though extensive membrane damage due to shear forces have been reported to decrease the number of viable or intact cells.[6]

Vortex disaggregation involves placing a fresh tissue sample in RPMI, then vortexing or vigorously shaking the sample for approximately 10 seconds until the RPMI solution becomes cloudy. Larger specimens may be serially sectioned prior to disaggregation. Small samples, such as GI or skin biopsies may be vortexed in toto. Remaining tissue fragments are subsequently processed for routine histology while the single cell suspension is processed as per any other single-cell suspension for flow cytometric analysis. This method is useful for extremely small samples and "dry-tap" bone marrow biopsies. This is also the only method in which histologic and flow cytometric correlation can be performed on the same tissue sample. The vortex method has been reported to yield lower cell counts from bone marrow core biopsies or extremely small and/or fibrotic samples; however, these samples largely provided diagnostic results and correlated with histology findings.[6] As with essentially any tissue disaggregation technique, samples with significant amounts of necrosis and/or fibrosis remain a challenge.

Mechanical Automated Disaggregation (Commercial/Medimachine[5])

Automated methods using commercially available disaggregation machines offer a fast (typically <5 min processing time) and simple technique for creating single cell suspensions from tissue or

bone marrow samples. They require limited tissue handling and provide a closed disposable system with standardized, operator-independent procedures.

Figure 3 below illustrates the procedure for mechanical automated disaggregation using a commercially-available disaggregation machine.

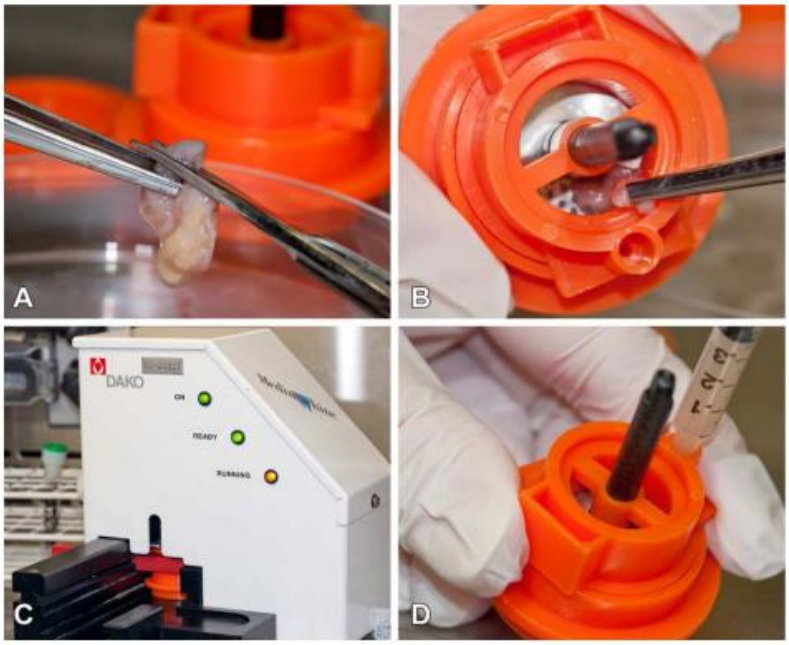


Figure 3 (from Reference 2). Automated method. (A) Slicing tissue into smaller fragments, (B) Placing tissue into commercially available disaggregation device, (C) Device in disaggregation machine, and (D) Cell suspension aspirated into tuberculin syringe.

Study results are mixed on the utility and yield of these types of automated mechanical disaggregation techniques. Novotny et al.[7] used this technique on non-aspirable bone marrow samples and found at least equivalent yield compared to the vortex technique described by Vos et al.[6] However, other studies have demonstrated the limitations of the automatic mechanical disaggregation method (see histograms below in Figure 4) and obtained higher percentages of diagnostic flow samples utilizing manual techniques, particularly in large cell lymphomas.[2, 6]

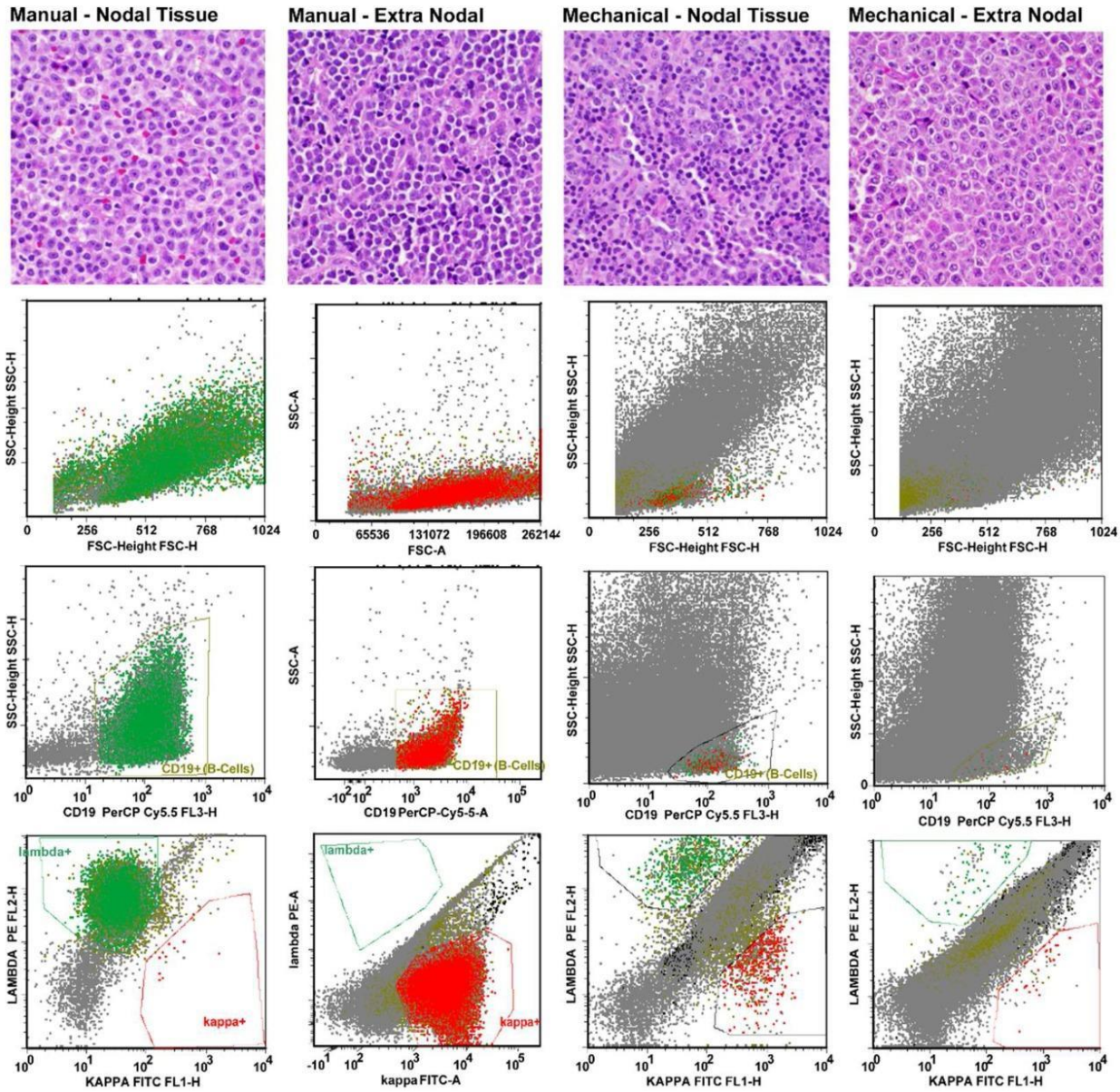


Figure 4 (from Reference 2). Comparison of flow histograms obtained using manual versus automatic tissue disaggregation.

Table 1. Summary Table of Advantages and Disadvantages.

Technique	Advantages	Disadvantages
Needle, Mesh, Scraping, Mincing, Tissue press	1) Gentler tissue processing 2) Higher cell yield 3) Decreased cell debris	1) Increased tissue handling 2) Limited by necrosis and fibrosis
Vortex	1) Allows histologic and flow cytometric correlation on same tissue sample 2) Well-suited for extremely small samples 3) Minimal tissue handling 4) For use with tissue and non-aspirable bone marrow biopsies	1) Limited by necrosis and fibrosis
Medimachine	1) Commercially available 2) Standardized 3) Operator-independent 4) Closed disposable system	1) High shear forces 2) Particularly susceptible to loss of large cell lymphomas

VIABILITY

Assessment of cellular viability is essential for accurate flow cytometric analysis and may be a good way to demonstrate the effectiveness of your preferred tissue disaggregation technique. Non-viable cells can demonstrate non-specific antibody binding of fluorescent antibodies and/or display unusual autofluorescence that can interfere with identification of abnormal populations. In addition, in quantitative assays such as lymphocyte enumeration (T-cell subsets) or CD34+ stem cell counts, the presence of non-viable cells can cause inaccurate results. Thus, the purpose of viability assessment is to ensure sample integrity, allow for precise measurement of antigenically-intact (viable) cells, and quantitation and/or exclusion of non-viable cells. In addition, CAP accreditation requires "a policy for determining when the percentage of viable cells in each test specimen should be measured" (refer to the CAP Flow Cytometry Checklist; FLO.30610).[8-9]

Cellular viability is often also closely related to specimen type. For example, fresh peripheral blood samples normally demonstrate excellent viability, which decreases over time. However, decreased cell survival is common in tissue samples and increases in high-grade neoplasms with high cellular turnover or necrosis or those exposed to tissue processing or prolonged storage.

There are several methods for assessment of cell viability. The two most commonly used fluorescent viability dyes are propidium iodide (PI) and 7-aminoactinomycin D (7-AAD)[10]. Both of these dyes intercalate with (attach or insert into) DNA base pairs meaning that cellular staining (positivity) is indicative of a compromised cellular membrane. Both dyes are excited by a blue

laser (488 nm) with emission maxima of 617 nm (PI) and 647 nm (7-AAD).[8-10] Gating on viable events is performed by drawing a gate around the PI or 7-AAD negative events on viability dye versus side scatter dot plots. A rough estimate of viable cells can also be approximated by excluding low forward scatter (FSC) and high side scatter (SSC) events (see Figure 5 below).[10]

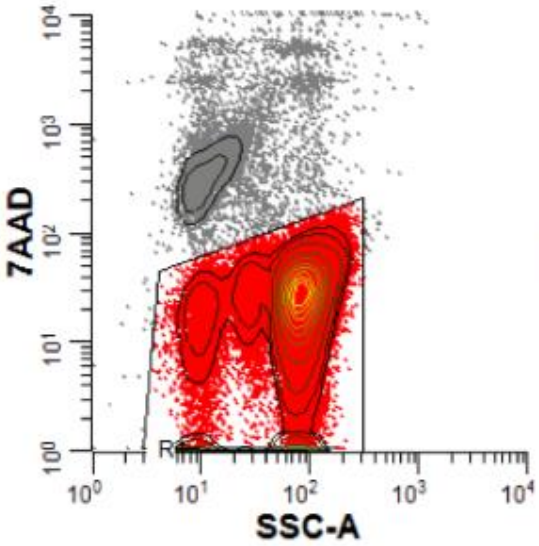


Fig. 1: Viability gating with 7-AAD

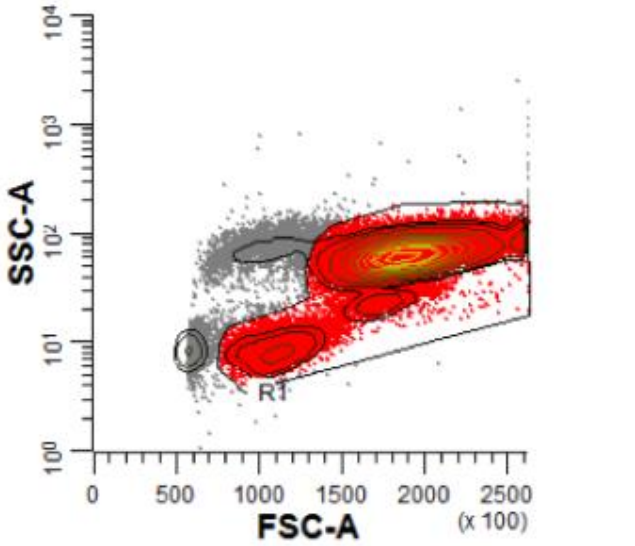


Fig. 2: Non-viable cell exclusion by FSC/SSC

Figure 5 (from reference 9). Viability gating

SUMMARY

Tissue disaggregation remains one of the most important and problematic steps in solid tumor analysis by flow cytometric immunophenotyping. The method used for tissue disaggregation can have a definite impact on cell loss, viability, non-specific antibody binding, and antigenicity. There are several options for performing tissue disaggregation available as discussed above. The goal here is not to present a single optimal approach, but to provide information regarding the advantages and disadvantages of each technique.

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9. ICCS Ask an Expert by Adam Seegmiller "How and in what situations should viability of specimens for flow cytometry be assessed?"
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For any questions on this module or any other suggestions, please email info@cytometry.org

Reviewed and approved by: George Deeb, MD and Jean Oak, MD