

HANDLING PLEURAL FLUID SAMPLES FOR ROUTINE ANALYSES

RUTİN ANALİZLER İÇİN PLEVRAL SIVI ÖRNEKLERİNİN KULLANIMI

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Abstract

Diagnostic thoracentesis requires approximately 20 to 40 mL of pleural fluid. The fluid should be placed in EDTA- or heparin-treated tubes for biochemical, microbiological, and cytological analyses when appropriate. The additional use of blood culture bottles improves bacterial isolation. Ideally, fluid specimens should be sent immediately to the laboratory and processed within 2 hours. If a delay is expected, the sample should be maintained at 4°C until analysis, except for microbiological cultures. A delayed analysis of more than 48 hours is unacceptable, although the cytomorphological features of refrigerated samples are well preserved for at least 72 hours.

Key words: Pleural fluid, pleural effusion, specimen handling

The removal of a small volume of pleural fluid for analysis (diagnostic thoracentesis) is performed to identify the cause of an effusion. The proper collection and handling of fluid specimens is of paramount importance as it may affect the reliability and accuracy of analyses. Inadequately trained clinicians sometimes send a single vacuum bottle or large syringe to be circulated through various laboratory sections or even different laboratories. Often, a sample may contain a large blood or fibrin clot as the result of inadequate anticoagulation or mixing. This article briefly describes the optimal conditions for the collection, transportation, and processing of pleural fluid samples in routine clinical practice, thus avoiding potential sources of preanalytical errors.

Özet

Tanısal torasentez yaklaşık 20 ila 40 mL plevral sıvı gerektirir. Uygun olduğunda sıvı; biyokimyasal, mikrobiyolojik ve sitolojik analizler için EDTA -veya heparin- ile muamele edilmiş tüplere konmalıdır. Ek olarak kan kültürü şişelerinin kullanımı bakteriyel izolasyonu artırır. İdeal olarak, sıvı örnekleri laboratuvara hemen gönderilmelidir ve 2 saat içinde işleme alınmalıdır. Bir gecikme bekleniyorsa örnek, mikrobiyolojik kültür haricinde, analize kadar 4°C'de muhafaza edilmelidir. Buzdolabındaki örneklerin sitomorfolojik özellikleri en az 72 saat için iyi korunuyor olmakla beraber 48 saatten daha fazla gecikmiş analiz kabul edilemez.

Anahtar kelimeler: Plevral sıvı, plevral efüzyon, örnek kullanımı

Obtaining Pleural Fluid

Pleural fluid specimens are obtained with a needle and syringe under aseptic conditions. The syringe and needle used to inject the local anaesthetic (e.g., 2% mepivacaine or lidocaine) into the epidermis and parietal pleura should not be used to obtain the pleural fluid sample (1). Other than the issue of dilution, the acidic local anaesthetic might artificially lower the pH of the aspirated pleural fluid (2). In small parapneumonic effusions, this could lead to a failed prediction as to whether a chest tube will be necessary (2).

Specimen Collection

Between 20 to 40 mL of pleural fluid is needed for a complete analysis, which includes biochemical, cyto-

logical and, when an infection is suspected, microbiological studies (3). Specimens should be collected in tubes containing anticoagulants, such as ethylenediaminetetraacetic acid (EDTA; lavender top) or heparin (green top), in order to avoid clotting and cell clumping, which may give inaccurate cell counts and differentials. In one study, leukocyte cell counts from plain tubes (i.e., without anticoagulant) were significantly lower (by about 50%) than those obtained from EDTA-treated tubes (4).

The recommended distribution of pleural fluid for various analyses is outlined in Table 1. One 5 mL tube is enough for biochemical analysis. For pH measurements, it has traditionally been taught that fluid should be collected anaerobically in a heparinised syringe and submitted to the laboratory on ice (5). More commonly, the fluid sample is obtained using an unheparinised syringe and then transferred to a heparinised tube. This manoeuvre does not cause a clinically relevant increase in pH, provided care is taken to avoid significant exposure to air (6). Nor is it necessary to pack the fluid sample in ice, as long as the pH determination is performed within 4 hours following extraction (7,8). Ideally, the measurement should be obtained using a blood gas machine. In grossly purulent specimens, the measurement of pH or any assay, other than a Gram stain and culture, is of no value and should not be conducted as the clinical decision is straightforward; that is, chest drainage is required for empyemas regardless of pleural pH value (3).

Plus, pus may clog and damage the analyser. Factors influencing pleural fluid pH have less effect on glucose concentration, suggesting that the latter can be used as an acceptable alternative to the former (8).

If microbiological analysis is indicated, inoculation of 2 to 5 mL pleural fluid into aerobic and anaerobic blood culture bottles, in addition to sending samples in a sterile tube for Gram stain and standard processing, increases the sensitivity of bacterial cultures by 20% (9). For pleural tuberculosis, the inoculation of 5 mL into liquid culture media is as beneficial (50% positivity) as the use of larger volumes (10).

While the volume of pleural fluid necessary for the maximum yield of cytological analysis is controversial, some guidelines suggest submitting at least 20 mL (11). The larger the sediment to be obtained in order to maximise the yield of cell blocks, the greater the volume of fluid that needs to be centrifuged.

Pleural Fluid Transportation and Processing

Fresh fluid should be promptly transported to the laboratory at ambient temperature. The maximum acceptable time delay before the processing of pleural fluid specimens in the laboratory is 2 hours (5). If a longer delay is expected, the specimen should be stored in the refrigerator at 4°C, except for microbiological cultures. Refrigerated storage for up to 48 hours has no significant effect on the total leukocyte count or differential, biochemical parameters (except lactate de-

Table 1. Routine tests for the evaluation of pleural effusions

Test	Submission method	Volume, mL	Comments
Cell count and differential	Heparin- or EDTA-treated tube	3-5*	Modern automated analysers are as reliable as microscopic analysis
pH	Heparinised syringe (e.g., arterial blood gas syringe) or tube Optional transportation on ice	2*	Admixture of sample with local anaesthetic, exposure to air, use of certain analysers, or delayed analysis may cause significant alterations in the pH value
Biochemistries	Heparin- or EDTA-treated tube, or tube without additives	3-5*	Routine fluid chemistries include protein, lactate dehydrogenase, glucose and, if available, adenosine deaminase
Cytological profile	Heparin-, EDTA-, or citrate-treated tube or container	10-20	Yield increases if cell blocks and immunocytochemistry, in addition to smears, are evaluated
Gram stain and bacterial cultures	Heparin- or EDTA-treated tube, along with blood culture bottles	5-10	Nucleic acid amplification tests for different microorganisms, as well as pneumococcal antigen tests, can be performed
Mycobacterial cultures	Heparin- or EDTA-treated tube	5	Liquid media are more sensitive than conventional solid media

* A total volume of 3–5 mL is enough for the sequential measurement of pH, cell counts, and biochemistries

hydrogenase [LDH], which may experience a reduction in concentrations from day 2 onward), or cytomorphology (12,13). Conversely, refrigeration inhibits the viability of certain microorganisms and, therefore, should be discouraged for specimens sent for microbial studies. In any case, delays of over 48 hours are unacceptable.

Automated multichannel analysers are used to assess pleural biochemical parameters. Firstly, the anticoagulant-coated tube containing the fluid sample is used for measuring both pH in a blood gas analyser, and total (red blood cells and leukocytes) and differential (polymorphonuclear vs. mononuclear leukocytes) cell counts in an automated cell counter (or less frequently, manually) after gentle agitation. Then, the tube is centrifuged at 3000 rpm for 15 minutes at room temperature, and the supernatant is tested for protein, LDH, glucose, and adenosine deaminase. Optional pleural fluid biochemistries include albumin and cholesterol (suspicion of misclassified transudate), amylase (pancreatitis or oesophageal rupture), triglycerides (chylothorax), C-reactive protein (bacterial infection), and tumour markers (malignancy) (3).

In the microbiology laboratory, Gram stain and both aerobic and anaerobic cultures should be set up. After high-speed centrifugation of the specimen (e.g., 3000 rpm x 15 minutes), the sediment is used to prepare the smears (Gram stain and acid fast stain for *Mycobacterium spp.*) and inoculate the culture media. Usually, blood agar, chocolate agar, MacConkey agar and, if the sample has been properly obtained, fastidious anaerobic agar plates are prepared, incubated at 35-37°C and monitored for microbial growth for a minimum of 4 days. Alternatively, blood culture bottles containing pleural fluid are incubated and, if positive, subcultures are then performed on the appropriate solid media to identify the microorganism (9). For mycobacterial identification, the use of liquid culture-based techniques, such as BACTEC and microscopic-observation drug-susceptibility (MODS) systems, provides higher yields and faster results than solid conventional media (Lowenstein-Jensen) (14).

As far as cytological examinations are concerned, it has been demonstrated that cellular integrity is well preserved for up to 72 h with appropriate refrigeration (2-8°C) (13). The routine cytopathological examination of pleural fluid involves the preparation of both cytospin smears and cell blocks. There are various fixation and staining techniques for cytospin smears and cell blocks prepared from the sediment after centrifugation. Slides are usually fixed in 95% isopropyl alcohol and stained with the Papanicolaou method, which clearly brings

out nuclear details, thus allowing better identification of malignant cells (15). Moreover, cell blocks fixed in formalin are stained with haematoxylin and eosin, and represent the ideal specimens for immunocytochemistry. A little-known fact is that cytospin material, including previously Pap-stained slides, can also be used for immunocytochemical analysis. In this author's experience, limited immunocytochemical panels that include epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), calretinin, and thyroid transcription factor-1 (TTF-1) are especially useful for the differential diagnosis between reactive mesothelial cells, adenocarcinoma, and mesothelioma (16).

Sample Storage for Research Purposes

There is scarce information on the stability of pleural fluid components after prolonged storage. For future research studies, it is recommended that the fluid sample be centrifuged and the supernatant and/or pellet be stored at -80°C. In our experience, as far as protein, LDH, and ADA are concerned, pleural fluid samples are viable for at least a few years (17). Similarly, in one study, commonly tested cytokines were highly reproducible when repeated at a median of 45 days apart, and showed stability following repeated freeze-thaw cycles (18).

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