Q&A column

Editor: Frederick L. Kiechle, MD, PhD

Submit your pathology-related question for reply by appropriate medical consultants. CAP TODAY will make every effort to answer all relevant questions. However, those questions that are not of general interest may not receive a reply. For your question to be considered, you must include your name and address; this information will be omitted if your question is published in CAP TODAY.

Q. What is the recommended procedure for analyzing cerebrospinal fluid from patients suspected of having Creutzfeldt-Jakob disease? In addition to sending the specimen to the National Prion Disease Pathology Surveillance Center for 14-3-3 testing, should the laboratory perform a cell count and/or meningitis panel?

A.April 2021—Obtaining cerebrospinal fluid from patients with clinical suspicion of prion diseases is important for the workup of these diseases. In conjunction with imaging studies and, sometimes, electroencephalography, using CSF to look for 14-3-3 protein changes and for testing with real-time quaking-induced conversion (RT-QuIC) can help distinguish prion diseases from other neurodegenerative, infectious, or autoimmune mimics, according to recent reports.[1,2] The reports demonstrate a higher sensitivity and specificity with RT-QuIC than 14-3-3. Both tests can be ordered through the National Prion Disease Pathology Surveillance Center or commercial vendors.

It may also be important to submit CSF for cell count, cytologic evaluation, and other studies if infections or neoplastic disease are part of a differential diagnosis.

1. Rudge P, Hyare H, Green A, Collinge J, Mead S. Imaging and CSF analyses effectively distinguish CJD from its mimics. *J Neurol Neurosurg Psychiatry*. 2018;89(5):461–466.
2. Franceschini A, Baiardi S, Hughson AG, et al. High diagnostic value of second generation CSF RT-QuIC across the wide spectrum of CJD prions. *Sci Rep*. 2017;7(1):10655.

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Q. Is light protection needed for folate samples? Most major reference laboratories do not require

folate samples to be protected from light, and I could not find any studies on the topic.

A.Folate is a form of vitamin B9 that is essential for DNA synthesis and multiple metabolic reactions. It may be useful to measure serum folate in the workup of anemia and evaluation of nutritional status. Studies have demonstrated that ultraviolet light can deplete folate in vivo and in vitro and that this process depends on other components in the blood that act as photosensitizers and photoprotectors.[1] Assay instructions from several manufacturers state that folate samples should be protected from light but often do not include a reference for the recommendation.

There is limited and conflicting evidence regarding whether exposing blood tubes to light would have a significant impact on subsequent folate measurements. Komaromy-Hiller, et al., found no significant differences in folate results with up to three days of storage in light-protected and light-exposed conditions.[2] They concluded that folate may be light sensitive in pure form but not in serum and that serum-binding proteins may have a protective effect. More recently, Huguenin, et al., concluded that photoprotection was not necessary if the sample was measured within four hours of collection. However, the study did not involve testing after four hours.[3] Clement and Kendall performed a study of light-protected versus light-exposed folate samples across seven days and found no more than a 1.7 percent decline in assayed values and no significant difference between storage conditions.[4] Collectively, the study results suggest that routine protection from light is not necessary if folate testing is performed on site and samples are analyzed consistently within a few hours of collection.

If folate is sent to a reference laboratory, light protection may not be necessary if the sample is analyzed within a few days. Samples transported to reference laboratories in closed refrigeration or freezer boxes likely do not need special light protection (e.g. foil, wrap, or amber tubes), as short periods of light exposure pre- and post-packaging should not have significant effects on folate results. However, laboratories should defer to their respective reference laboratory specimen requirements or manufacturer package inserts. Deviating from manufacturer instructions converts the assay into a laboratory-developed test, necessitating that the assay be validated appropriately.

1. Juzeniene A, Thu Tam TT, Iani V, Moan J. 5-methyltetrahydrofolate can be photodegraded by endogenous photosensitizers. *Free Radic Biol Med*. 2009;47(8):1199–1204.
2. Komaromy-Hiller G, Nuttall KL, Ashwood ER. Effect of storage on serum vitamin B12 and folate stability. *Ann Clin Lab Sci*. 1997;27(4):249–253.
3. Huguenin A, Oudart JB, Hubert J, Maquart FX, Ramont L. Serum folate and vitamin B12: does light really matter? *Clin Chem Lab Med*. 2014;52(9):e203–e204.
4. Clement NF, Kendall BK. Effect of light on vitamin B12 and folate. *Lab Med*. 2009;40(11):657–659.

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Q. Many times a platelet count on an automated hematology system indicates some degree of thrombocytopenia or the analyzer reports a high mean platelet volume or platelet large cell ratio, while a blood smear shows large platelets and/or giant platelets. Is it OK to include a comment in the report that the platelets are adequate or that the count could be due to large platelets, especially with values that indicate marked thrombocytopenia?

A.Modern automated hematology analyzers usually provide accurate platelet counts, especially hematology analyzers that, in addition to using electrical impedance, use alternative methods for counting platelets, such as optical technology (light scatter or fluorescent flow cytometry) or immunologic methods. Furthermore, computer algorithms for modern hematology analyzers recognize interference or an abnormal platelet distribution.

Automated hematology analyzers will flag an automated platelet count for quantitative changes, such as when the platelet count is below or above a laboratory-defined cutoff on a new patient or delta checks show significant variation in platelet count. They will also flag an automated platelet count for qualitative changes, such as platelet clumps, abnormal platelet distribution, giant or large platelets, red blood cell fragments, or an abnormal platelet scattergram.

When a platelet count is flagged, it is important to verify the count by estimating platelets from a well-prepared peripheral blood smear. This is necessary since inaccuracy can be due to platelet characteristics that overlap those of other cellular material, such as schistocytes and leukocyte cytoplasmic fragments, the presence of cryoglobulins, and the inherent ability of platelets to activate and clump. In addition, giant platelets may not be counted by automated analyzers because their size exceeds the normal threshold value. A platelet estimate from a blood smear is an acceptable method for counting platelets. Each laboratory should develop an adequate system for correlating automated platelet counts with manual microscopic counts to prevent reporting spurious thrombocytopenia (due to platelet clumps, giant platelets, or platelet satellitism) or thrombocytosis (due to microcytic red blood cells, cytoplasmic fragments, fungal or bacterial organisms, debris, or electronic noise).

To verify the platelet count, the entire blood smear, including the feather edge, lateral edges, readable area, and thick area, should be examined under low magnification for the presence of clumps of platelets. The blood smear should then be examined under higher magnification for the presence of red cell fragments, bacterial or fungal organisms, debris, and giant platelets. If any of these interferences are present, the automated platelet count is unreliable, and a platelet scan comment should be reported in qualitative terms as normal, increased, or decreased. The comment should also mention the type of interference—for example, “normal platelet count with giant platelets present” or “normal platelet count with red cell fragments present.”

If platelets are clumped after collection in an EDTA-anticoagulated tube that was well mixed at the time of collection, this may represent in vitro EDTA-induced changes. Platelets must be quantified from blood collected directly into a counting diluent using the anticoagulant recommended by the manufacturer of the counting diluent or by estimating the count from a non-anticoagulated blood film.

D’Souza C, Briggs C, Machin SJ. Platelets: the few, the young, and the active. *Clin Lab Med*. 2015;35(1):123–131.

Gulati G, Song J, Florea AD, Gong J. Purpose and criteria for blood smear scan, blood smear examination, and blood smear review. *Ann Lab Med*. 2013;33(1):1–7.

Segal HC, Briggs C, Kunka S, et al. Accuracy of platelet counting haematology analysers in severe thrombocytopenia and potential impact on platelet transfusion. *Br J Haematol*. 2005;128(4):520–525.

Tantanate C, Khowawisetsut L, Pattanapanyasat K. Performance evaluation of automated impedance and optical fluorescence platelet counts compared with international reference method in patients with thalassemia. *Arch Pathol Lab Med*. 2017;141(6):830–836.

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