

Submission ID #: 71690

Scriptwriter Name: Sri Lakshmi Priya

Project Page Link: <https://review.jove.com/account/file-uploader?src=21865498>

Title: Workflow for Automated Digital Peripheral Blood and Bone Marrow Cytomorphology, With Consideration of Critical Cellular Misclassifications

Authors and Affiliations:

David Starostka¹, Richard Dolezilek², Hans Michael Kvasnicka³, Barbora Sotkowska¹, Petra Miczkova¹

¹Laboratory of Hemato-oncology and Clinical Biochemistry, Moravian-Silesian Hospital Havírov

²Department of Pathology, Moravian-Silesian Hospital Havírov

³Institute of Pathology and Molecular Pathology, University Clinic Wuppertal

Corresponding Authors:

David Starostka david.starostka@nemhav.cz

Email Addresses for All Authors:

David Starostka	david.starostka@nemhav.cz
Richard Dolezilek	richard.dolezilek@nemhav.cz
Hans Michael Kvasnicka	hansmichael.kvasnicka@helios-gesundheit.de
Barbora Sotkowska	barbora.sotkowska@nemhav.cz
Petra Miczkova	petra.miczkovalnemhav.cz

Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES, done**
- 3. Filming location:** Will the filming need to take place in multiple locations? **NO**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **NO**

Current Protocol Length

Number of Steps: 32

Number of Shots: 58

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Authors: *Answers are slightly edited in accordance with journal guidelines. Please note that the questions will not appear on screen. Please answer in **stand-alone full sentence with sufficient context***

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Richard Dolezilek:** Our research presents an automated digital cytomorphology workflow for peripheral blood and bone marrow, focusing on critical misclassifications.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Richard Dolezilek:** Correct cytomorphological assessment remains a cornerstone of multidisciplinary hemato-oncology diagnostics, and this automated digital cytomorphology protocol complements comprehensive diagnostic integration.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **David Starostka:** This protocol allows users to exploit the benefits of automated cytomorphology through its workflow and expert review process.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **David Starostka:** The most important challenge is the proper classification of neoplastic lymphocytes, small blasts, granular monoblasts, dysplastic promonocytes, monocytes, and immature plasma cells.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. David Starostka: Future studies can build on this work through multi-institutional validation across different clinical settings and model optimization using data augmentation strategies.
- 1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Local Ethics Committee at Hospital Havířov. Written consent was obtained from all the participants

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Automated Peripheral Blood Smear Preparation and Staining

Demonstrator: Petra Miczkova

2.1. To begin, wear latex or nitrile gloves before handling peripheral blood and bone marrow samples and smears [1]. Check the tubing condition [2], network connection [3], and waste container status [4]. Confirm that sufficient reagents and an adequate supply of microscope slides are available [5].

2.7.1, Talent putting on latex or nitrile gloves **Author's NOTE: Move 2.71 above 2.1.1**

2.1.1. WIDE: Talent inspecting the tubing condition. ***Videographer:** Please obtain CU shot as well for this shot*

2.1.2. Talent checking the network connection.

2.1.3. Talent inspecting the waste container status.

2.1.4. Talent arranging the reagents and microscope slides on the workbench.

2.2. Check that the spreader glass is intact, completely clean, and securely placed in the holder [1]. Turn on the instrument [2]. Allow the main unit to perform the automatic self-check [3].

2.7.4, Talent inspecting the spreader glass in the holder. **Author's NOTE: Move 2.7.4 above 2.2.1**

2.2.1. Talent turning on the instrument.

2.2.2. SCREEN: 71690_screenshot_01A.mp4: 00:00-00:08,
71690_screenshot_01B.mp4: 00:00-00:03

2.3. Log in to the software using a valid username and password and operate the instrument using the current software versions [1].

2.3.1. SCREEN: 71690_screenshot_02.mp4: 00:00-00:12

2.4. Select operations from the **Menu** [1]. Manage requests for slide preparation and smear preparation in the **Worklist** [2]. Review request data in the **Browser** [3]. Check the smear preparation status in the **Status** tab [4].

2.4.1. SCREEN: 71690_screenshot_03.mp4: 00:00-00:10, 00:14-00:25

2.4.2. SCREEN: 71690_screenshot_04.mp4: 00:00-00:10

2.4.3. SCREEN: 71690_screenshot_05.mp4: 00:00-00:10

- 2.4.4. SCREEN: 71690_screenshot_06.mp4: 00:00-00:05
- 2.5. Download a new slide preparation request from the host computer or create a new request [1].
 - 2.5.1. SCREEN: 71690_screenshot_07.mp4: 00:03-00:07, 00:11-00:32, 00:36-00:40, 01:12-01:15
- 2.6. Edit or delete slide preparation requests as needed [1].
 - 2.6.1. SCREEN: 71690_screenshot_08.mp4: 00:00-00:20
- 2.7. ~~Wear latex or nitrile gloves before handling peripheral blood and bone marrow samples and smears [1].~~ Select the smear preparation mode [1]. Place the sample rack into the sampler [2]. Start smear preparation and staining from the dialog window [3].
 - 2.7.1. ~~Talent putting on latex or nitrile gloves.~~ **NOTE: 2.7.1 and 2.7.4 are moved above and Vo is moved too**
 - 2.7.2. SCREEN: 71690_screenshot_09.mp4: 00:00-00:08
 - 2.7.3. Talent placing the sample rack into the sampler.
 - 2.7.4. ~~Talent inspecting the spreader glass in the holder.~~ **NOTE: 2.7.1 and 2.7.4 are moved above and Vo is moved too**
 - 2.7.5. SCREEN: 71690_screenshot_10.mp4: 00:15-00:21
- 2.8. Check the status of the smear being prepared and review the details of the slide preparation requests [1].
 - 2.8.1. SCREEN: 71690_screenshot_11.mp4: 00:13-00:19, 01:09-01:13, 01:32-01:37
- 2.9. Remove the sample rack [1] and the prepared and stained slides [2].
 - 2.9.1. Talent removing the sample rack.
 - 2.9.2. Talent removing the prepared and stained slides.
- 2.10. After performing the instrument inspection and cleaning, turn off the instrument [1].
 - 2.10.1. Talent turning off the instrument.

3. Automated Peripheral Blood Smear Analysis

Demonstrator: Barbora Sotkovska

- 3.1. Once the analyzer is started, log in to the software using a valid username and password and operate the instrument using the current software versions [1].
 - 3.1.1. SCREEN: 71690_screenshot_12.mp4: 00:00-00:16

- 3.2. Open the New Analysis window and load the prepared peripheral blood smear into the analyzer [1].
 - 3.2.1. SCREEN: 71690_screenshot_13.mp4: 00:00-00:25
- 3.3. Perform the daily internal quality control using a control slide [1]. Run the cell detection test once daily to detect at least 100 cells [2]. Perform cell detection test [3].
 - 3.3.1. Talent placing the control slide into the analyzer.
 - 3.3.2. SCREEN: 71690_screenshot_14.mp4: 00:00-00:17
 - 3.3.3. SCREEN: 71690_screenshot_15.mp4: 00:00-00:13
- 3.4. Prepare the peripheral blood smear with the slide-making and staining instrument [1]. Place the stained smear into the analyzer loading drawer [2].
 - 3.4.1. Talent holding the prepared peripheral blood smear.
 - 3.4.2. Talent placing the stained smear into the analyzer loading drawer.
- 3.5. Enter the test identification manually or with a barcode scanner and close the analyzer input door [1].
 - 3.5.1. SCREEN: 71690_screenshot_16.mp4: 00:00-00:18
- 3.6. Start the slide processing [1].
 - 3.6.1. SCREEN: 71690_screenshot_16.mp4: 00:22-00:26
- 3.7. Review the preliminary results [1]. Reclassify cells as necessary on the analyzer or using the remote review software, considering the three proposed classification options and their corresponding probability percentages [2].
 - 3.7.1. SCREEN: 71690_screenshot_18.mp4: 00:03-00:07
 - 3.7.2. SCREEN: 71690_screenshot_19.mp4: 00:00-00:16
- 3.8. In WBC mode, review the preliminary classification of nucleated cells [1]. Reassign the cell by dragging its image to the correct category if an incorrect classification is identified [2].
 - 3.8.1. SCREEN: 71690_screenshot_20-HORIZONTAL.mp4.
 - 3.8.2. SCREEN: 71690_screenshot_21.mp4: 00:10-00:20
- 3.9. In RBC mode, review the assessment of RBC morphological changes and correct inaccurate evaluations or add additional pathological features [1].
 - 3.9.1. SCREEN: 71690_screenshot_22.mp4: 00:00-00:07, 00:32-00:41
- 3.10. In platelet or PLT mode, review the platelet evaluation [1].
 - 3.10.1. SCREEN: 71690_screenshot_23: 00:00-00:06.
- 3.11. Add comments manually or select predefined text options [1]. Confirm the final result

for automatic transmission to the laboratory information system [2].

3.11.1. SCREEN: 71690_screenshot_24.mp4: 00:00-00:23

3.11.2. SCREEN: 71690_screenshot_25.mp4: 00:00-00:15

3.12. After logging out of the software, shut down the analyzer [1].

3.12.1. Talent shutting down the analyzer.

4. Automated Bone Marrow Differential Cell Analysis

Demonstrator: Katerina Chasakova

4.1. After starting the analyzer, log in to the analyzer software using a valid username and password and operate the instrument using the current software versions [1].

4.1.1. SCREEN: 71690_screenshot_27.mp4: 00:00-00:07, 00:21-00:25

4.2. Open the **Project** tab and review the processing orders [1]. After reviewing the template configuration, use **Edit** to configure classification rules, morphology, and the pre-classification strategy [2]. Following the report preview, add the findings and opinion, then generate the report [3]. Using the **Manage** tab, set the account settings and configure the signature rules [4].

4.2.1. SCREEN: 71690_screenshot_28.mp4: 00:03- 00:13, 00:22-00:35

4.2.2. SCREEN: 71690_screenshot_29.mp4: 00:38-00:47, 00:58-01:10, 01:20-01:29, 01:39-01:43

4.2.3. SCREEN: 71690_screenshot_30.mp4: 00:14-00:18, 00:42-00:50, 01:01-01:09

4.2.4. SCREEN: 71690_screenshot_31.mp4: 00:29-00:39, 00:43-00:48, 01:03-01:11

4.3. After reviewing the preliminary results, reclassify cells where necessary on the analyzer or using the remote review software, and reassign incorrectly classified cells to the correct category [1].

4.3.1. SCREEN: 71690_screenshot_32.mp4: 00:48-00:56, 04:15-04:29

4.4. Add comments and cytomorphological descriptions manually or by selecting predefined text options [1].

4.4.1. SCREEN: 71690_screenshot_33.mp4: 00:05-00:11, 00:52-01:09

Results

5. Results

5.1. Correctly classified cells generally displayed typical cytomorphological features [1], whereas incorrectly classified cells frequently showed atypical morphology [2].

5.1.1. LAB MEDIA: Figure 4A.

5.1.2. LAB MEDIA: Figure 5.

5.2. Misclassifications most often occurred between atypical lymphocytes and blasts [1], myeloblasts and lymphocytes [2], lymphoblasts and lymphocytes [3], monoblasts or promonocytes and promyelocytes [4], and immature plasma cells and blasts [5].

5.2.1. LAB MEDIA: Figure 4B. *Video editor: In the middle panel, highlight the rows where Atypical lymphocyte appears under Expert classification and Blast, NOC under ADM pre-classification.*

5.2.2. LAB MEDIA: Figure 5. *Video editor: Highlight the 2 rows with AML under diagnosis.*

5.2.3. LAB MEDIA: Figure 5. *Video editor: Highlight the rows with Lymphoblast under Expert classification and Lymphocyte under ADM classification.*

5.2.4. LAB MEDIA: Figure 5. *Video editor: Highlight the examples with Monoblast under Expert classification and Promyelocyte and Blast, NOC under ADM classification.*

5.2.5. LAB MEDIA: Figure 5. *Video editor: Highlight the row with Immature plasma cell under Expert classification and Blast, NOC under ADM classification.*

5.3. In lymphocytic neoplasms, representative examples of misclassified neoplastic lymphocytes were identified [1].

5.3.1. LAB MEDIA: Figure 5. *Video editor: In the left panel, highlight the rows labelled Splenic MZL, MCL, HCL, MZL, and CLL, pleomorphic variant.*

5.4. In acute leukemia and chronic myelomonocytic leukemia, representative examples showed misclassified lymphoblasts, myeloblasts, monoblasts, and promonocytes [1].

5.4.1. LAB MEDIA: Figure 5. *Video editor: In the right panel, highlight the rows labelled T-ALL and AML.*

5.5. In multiple myeloma, misclassifications occurred within the plasma cell lineage, particularly involving plasmablasts [1] and immature plasma cells [2].

5.5.1. LAB MEDIA: Figure 5. *Video editor: In the right panel, highlight the rows labelled*

Plasmablast under Expert Classification.

5.5.2. LAB MEDIA: Figure 5. *Video editor: In the bottom right panel, highlight the rows labelled Immature plasma cell under Expert Classification.*

5.6. Critical misclassifications of neoplastic lymphocytes [1], small lymphoblasts or myeloblasts [2], granular monoblasts may pose a risk of misdiagnosis [3].

5.6.1. LAB MEDIA: Figure 5. *Video editor: Highlight the rows showing atypical lymphocyte under Expert Classification.*

5.6.2. LAB MEDIA: Figure 5. *Video editor: Highlight the rows showing lymphoblast and myeloblast under Expert Classification.*

5.6.3. LAB MEDIA: Figure 5. *Video editor: Highlight the row showing the Monoblast under Expert Classification.*

5.7. Similar risks were associated with critical misclassifications of dysplastic promonocytes and monocytes [1], plasmablasts [2], and immature plasma cells [3].

5.7.1. LAB MEDIA: Figure 4. *Video editor: Highlight the rows showing Promonocyte and Monocyte under ADM pre-classification.*

5.7.2. LAB MEDIA: Figure 5. *Video editor: Highlight the rows showing Plasmablast under Expert Classification.*

5.7.3. LAB MEDIA: Figure 5. *Video editor: Highlight the rows showing Immature plasma cell under Expert Classification.*

1. Peripheral

Pronunciation link: <https://www.merriam-webster.com/dictionary/peripheral>

IPA: /pəˈrɪf(ə)rəl/

Phonetic Spelling: puh·rif·er·uhl

2. Nitrile

Pronunciation link: <https://www.merriam-webster.com/dictionary/nitrile>

IPA: /ˈnaɪ.trɪl/

Phonetic Spelling: ny·tryl

3. Smear

Pronunciation link: <https://www.merriam-webster.com/dictionary/smear>

IPA: /ˈsmɪr/

Phonetic Spelling: smeer

4. Reagents

Pronunciation link: <https://www.merriam-webster.com/dictionary/reagent>

- IPA: /rē'ājənt/
Phonetic Spelling: ree·ay·juhnt
5. Microscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscope>
IPA: /'maɪkrə'skoʊp/
Phonetic Spelling: my·kruh·skohp
 6. Analyzer
Pronunciation link: <https://www.merriam-webster.com/dictionary/analyzer>
IPA: /'anə,līzər/
Phonetic Spelling: an·uh·ly·zer
 7. Morphology
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>
IPA: /môr'fāləjē/
Phonetic Spelling: mor·fol·uh·jee
 8. Nucleated
Pronunciation link: <https://www.merriam-webster.com/dictionary/nucleated>
IPA: /'nu:kli,etɪd/
Phonetic Spelling: noo·klee·ay·tid
 9. Platelet
Pronunciation link: <https://www.merriam-webster.com/dictionary/platelet>
IPA: /'plāt-lət/
Phonetic Spelling: playt·lit
 10. Cytomorphological
Pronunciation link: No confirmed link found
IPA: /,sartʊ,mɔrfə'lə:dʒɪkəl/
Phonetic Spelling: sy·toh·mor·fuh·loj·i·kuhl
 11. Lymphocyte
Pronunciation link: <https://www.merriam-webster.com/dictionary/lymphocyte>
IPA: /'lɪm(f)ə,sart/
Phonetic Spelling: limf·uh·syt
 12. Lymphoblast
Pronunciation link: <https://www.merriam-webster.com/dictionary/lymphoblast>
IPA: /'lɪm(f)ə,blæst/
Phonetic Spelling: limf·uh·blast
 13. Myeloblast
Pronunciation link: <https://www.merriam-webster.com/dictionary/myeloblast>
IPA: /'maɪələ,blæst/
Phonetic Spelling: my·uh·luh·blast
 14. Monoblast
Pronunciation link: <https://www.merriam-webster.com/dictionary/monoblast>
IPA: /'mɑ:nə,blæst/
Phonetic Spelling: mon·uh·blast
 15. Promonocyte
Pronunciation link: No confirmed link found

- IPA: /proʊˈmɑːnəˌsaɪt/
Phonetic Spelling: proh·mon·uh·syɪt
16. Promyelocyte
Pronunciation link: <https://www.merriam-webster.com/dictionary/promyelocyte>
IPA: /ˌproʊˌmaɪələˈsaɪt/
Phonetic Spelling: proh·my·uh·luh·syɪt
17. Plasmablast
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasmablast>
IPA: /ˈplæzməˌblæst/
Phonetic Spelling: plaz·muh·blast
18. Neoplasm
Pronunciation link: <https://www.merriam-webster.com/dictionary/neoplasm>
IPA: /ˈnēɒˌplazəm/
Phonetic Spelling: nee·oh·plaz·uhm
19. Leukemia
Pronunciation link: <https://www.merriam-webster.com/dictionary/leukemia>
IPA: /luˈkiːmiə/
Phonetic Spelling: loo·kee·mee·uh
20. Myelomonocytic
Pronunciation link: No confirmed link found
IPA: /ˌmaɪələʊˌmɑːnəˈsaɪtɪk/
Phonetic Spelling: my·uh·loh·mon·uh·sit·ik
21. Dysplastic
Pronunciation link: <https://www.merriam-webster.com/dictionary/dysplastic>
IPA: /dɪsˈplæstɪk/
Phonetic Spelling: dis·plas·tik
22. Cytomorphological
Pronunciation link: No confirmed link found
IPA: /ˌsaɪtoʊˌmɔːrfəˈlɑːdʒɪkəl/
Phonetic Spelling: sy·toh·mor·fuh·loj·i·kuhl