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Supplemental Methods

Definitions (Committee 2019)

Band neutrophil

A band neutrophil is round to oval, 10-18 μm in diameter, with a nuclear to cytoplasmic (N:C) ratio of 1:1.5 to 1:2 and condensed nuclear chromatin. The nucleus is indented to more than half the distance to the farthest nuclear margin, but the chromatin is not condensed to a single filament (as is the defining feature of the fully mature neutrophil). The nucleus can assume many shapes: it can be band- or sausage-like; S-, C-, or U-shaped; and twisted or folded on itself. The cytoplasm is similar to that of other post-mitotic neutrophils, with specific granules predominating in an otherwise pale cytoplasm.

Segmented neutrophil

A segmented neutrophil has a similar size to a band neutrophil (i.e., 10 to 15 μm in diameter), as well as comparable shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). The N:C ratio is 1:3, and the nuclear chromatin is highly condensed. The nucleus is segmented or lobated (with a normal range of three to five lobes). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, dark, thread-like line. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from the band neutrophil.

Metamyelocyte

Metamyelocytes are approximately 10 to 18 μm in diameter. They are round to oval with an N:C ratio of 1.5:1 to 1:1. The nuclear chromatin is condensed, and the nucleus is indented to less than half of the maximal nuclear diameter (i.e., the indentation is smaller than half of the distance to the farthest nuclear margin). The cytoplasm is amphophilic, containing rare azurophilic or purple (primary) granules and many fine lilac or pale orange/pink specific granules.

2020-A Mailing Survey Questionnaire De-Duplication Process

Survey respondents who provided complete responses to questions #1 (*What automated WBC differentials are reported by your instrument?*) or question #2 (*Does your laboratory perform manual differentials?*) were included in the initial analysis (n = 7,880, pre-de-duplication). Those who answered “yes” to question #2 (*Does your laboratory perform manual differentials?*) were instructed to answer all survey questions, while those who responded “No” to question #2 above, were instructed to skip questions #3 to #8 pertaining to band reporting but answer the remaining questions.

Given that the same laboratory could participate in multiple surveys and therefore answer the supplemental questions multiple times, the following de-duplication process was employed to ensure that each laboratory would provide only one set of laboratory practice answers to the questions. If there were identical responses from the same laboratory, then only one set of answers was taken. If there were different sets of responses from the same laboratory, the chosen responses were those from the questionnaire with an answer set of “Yes” to Survey Question

(SQ) #2 (*Does laboratory perform manual differentials?*) and the highest category of manual differentials to SQ3 (*On average, what percentage of all reported differentials are manual differentials?*). The final number of unique laboratories after de-duplication was 6,444, with 1,436/7,880 (18.2%) duplicate responses removed. The only addition of data was to make SQ2="Yes" when SQ2 was left blank or was answered "No", yet there were responses to SQ3 or SQ4 (*Who performs manual differentials in laboratory?*) (n=117). Responses to specific supplemental questions were excluded as defined by the skip sequence of the questionnaire. Specific removal of data fields included: when SQ2="No" (n=27), removal of SQ5 (*Does your laboratory use a digital imaging morphology system?*), SQ6 (*Does your laboratory report band neutrophil counts/percentages on manual differentials?*), and SQ8 (*Does laboratory provide a reference range when reporting band counts?*); when SQ6="No – never" (n=51), removal of SQ7 (*If your laboratory reports bands for specific patient populations, clinical scenarios, and/or at clinician request, which departments send the specimens?*) and SQ8; and if SQ6="Yes – always," (n=1,154) removal of multiple responses for SQ7.

Linkage of Cell Performance Identification in Virtual Peripheral Blood Smear (VPBS)-2020-B mailing to the 2020-A Questionnaire

Of the 1,328 responses from VPBS-B, 1,180 (88.9%) had a matching set of supplemental questions for SQ1, SQ2, SQ9, SQ10 and SQ11 from 2020-A. For SQ3, SQ4, SQ5 and SQ6, 1,165 cell ID performance data were linked (15 supplemental sets of answers were removed given an answer of "No" to SQ2). These performance and supplemental datasets were used for further analysis and statistical testing.

VPBS-B-2020 Survey Questionnaire

Of additional interest was whether performance of band enumeration on the morphologic cell identification (ID) challenges was associated with certain laboratory characteristics based on survey responses. Thus, the same 11-question survey distributed in the 2020 A mailing was re-circulated in the 2020-B mailing of the VPBS challenge, with an additional question: *What age group does your facility serve? (choices: pediatric only; both adult and pediatric; or adult only)*. However, the responses to the VPBS-B-2020 survey questionnaire were not further used in the analysis, as the VPBS-A-2020 responses were of sufficient number for the purposes of the study.

Supplemental Results

Hematology laboratory workflow: use of automated instrument and digital imaging morphology systems, laboratory monthly volume of all Complete Blood Count (CBC) with differential (CBC/D) orders, and types of technologists performing manual WBC differentials (2020-A mailing)

Responses to survey questions of all laboratories (n = 6,444) are seen in **Table 1** and **Table 2**. A large majority of respondent laboratories (5,682/6,375; 89.1% overall) have instruments that are capable of reporting 5-part or 6-part automated WBC differentials (SQ#1). A small percentage of laboratories (892/5,286; 16.9% overall) use a digital imaging morphology system (like Cellavision, Cobas m611, EasyCell assistant, HemeCAM, NextSlide, Vision Hema, etc.) (SQ#5).

Laboratory monthly CBC/D testing volumes are variable. Some laboratories (710/6,154; 11.5% overall) performed less than 100 CBC/Ds per month. The number of laboratories that perform 100-499 monthly CBC/Ds were 1,247/6,154 (20.3%) and for 500-999 monthly CBC/Ds were

709/6,154 (11.5%). The largest proportion of monthly CBC/D volumes was a testing volume of 1,000-4,999 CBC/Ds (1,799/6,154; 29.2% overall). Larger testing volumes were reported in fewer laboratories: 5,000- 9,999 CBC/Ds in 804/6,154 (13.1%); 10,000-14,999 in 383/6,154 (6.2%); and >15,000 monthly CBC/Ds in 502/6,154 (8.2%). The highest test volumes (>15,000 monthly) were reported in university/academic laboratories (22.4% of university labs) and national/reference laboratories (17.6% of national/reference laboratories).

Additional results on band reporting practice in the United States (US), Canada, and other countries (2020-A mailing)

Among the laboratories in the US that sometimes report bands per policy and/or at the request of clinical departments (n = 341 of 4,130 respondents; 8.3%), the top requestors for band reporting are “other” departments not otherwise specified (119/341; 34.9%) and neonatal intensive care units (ICU) (112/341; 32.8%). The departments of adult hematology/oncology (101/341;29.6%), emergency department and/or urgent care (101/341; 29.6%), and general pediatrics (98/341;28.7%) closely follow at similar frequencies.

For CAP-participating laboratories in Canada, the proportion of laboratories that sometimes report bands per policy and/or at clinical request is 49 of 410 Canadian respondents (12.0%). The top requesting departments in Canada are neonatal ICU (n = 22 of 49 respondents; 44.9%) and “other” departments not otherwise specified (n = 20 of 49 respondents; 40.8%), similar to the US. Interestingly, the adult hematology/oncology departments in Canada request band counting much less frequently than in the US (6.1% vs. 29.6%, respectively).

In non-US/non-Canada participating laboratories (n = 89 of 423; i.e. 21.0% of respondents) categorized as “other” countries, not otherwise specified), adult hematology/oncology is again

the top requesting clinical service (n = 44 of 89 respondents; 49.4%), with a significantly higher request rate as compared to US laboratories (n = 101 of 341; 29.6%) receiving requests from adult hematology/oncology departments. Most other services requesting at similar frequencies (range 30.3% to 38.2%, including general pediatrics, pediatric hematology/oncology, neonatal ICU, Emergency Department (ED)/urgent care, and critical care unit) [Table 4 and Figure 2].

Morphologic cell ID performance on intended responses associated with survey questionnaire responses (2020-A mailing)

The performance of laboratories for the intended morphologic cell IDs in the 2020-B mailing were linked to the survey questionnaire responses from the 2020-A mailing, with a final dataset of 1,165 laboratories (for SQ1, SQ2 and SQ9-SQ11) and 1,180 laboratories (for SQ3-SQ6), respectively, with cell ID performance and supplemental data used for further analysis and statistical testing (87.7% and 88.8%, respectively, of 1,328 responses) [Supplemental Table 2].

Statistical tests for differences in cell ID challenges with less than 85% agreement were performed against the different levels of eight selected SQs (cell IDs VDIFF-02, -06, -07, -08, and -12 tested against 8 SQs; thus, these 5 cell IDs were tested against 8 SQs = 40 tests). Since 40 specific tests were analyzed, a Bonferroni correction was applied to the critical value for these tests: $p\ 0.05/40 = p\ 0.0013$. Performance on morphologic cell ID was not significantly associated with most survey question responses, with the following exceptions: VDIFF07 (moderately challenging band) by SQ5 (digital imaging systems), SQ6 (band neutrophil reporting), and SQ9 (laboratory affiliation/practice setting); VDIFF08 (“easy” metamyelocyte) by SQ11 (laboratory location); and VDIFF12 (“easy” metamyelocyte) by SQ6 (band neutrophil reporting). Although the associations display numeric statistical significance (each, $p < 0.0013$), the absence of a consistent, logical pattern suggest that the associations occurred by chance. In other words,

differences in laboratory characteristics (namely, whether a laboratory performs high vs. low CBC/D testing volumes; whether generalist vs. specialist technologists review peripheral blood smears; whether a laboratory employs digital imaging systems; whether band neutrophils are reported; and laboratory practice setting) do not consistently predict better performance for the intended cell IDs.

Reference:

Committee, H. a. C. M. (2019). Hematology and Clinical Microscopy Glossary.

Abbreviations:

CAP – College of American Pathologists

CBC – complete blood count

CBC/D – complete blood count with differential

ED – emergency department

ICU – Intensive Care Unit

ID – identification

N:C ratio – nuclear to cytoplasmic

SQ – survey question

VPBS – virtual peripheral blood smear survey

WBC – white blood cells

US – United States

Supplemental Table 1. Survey questions (SQ) distributed to the College of American Pathologists program participants (2020-A mailing)

1. What automated WBC differentials are reported by your instrument?
 - a. 3 part (neutrophils, lymphocytes, mononuclear cells)
 - b. 5 part (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
 - c. 6 part (neutrophils, lymphocytes, monocytes, eosinophils, basophils, immature granulocytes)
2. Does your laboratory perform manual differentials?
 - a. No (Skip to question #9)
 - b. Yes (Proceed to question #3)
3. On average, what percentage of all reported differentials are manual differentials?
 - a. $\leq 5\%$
 - b. 6 – 10%
 - c. 11 – 15%
 - d. 16 – 20%
 - e. 21 – 25%
 - f. 26 – 30%
 - g. 31 – 35%
 - h. 36 – 40%
 - i. 41 – 45%
 - j. 46 – 50%
 - k. 51 – 60%
 - l. 61 – 70%
 - m. 71 – 80%
 - n. 81 – 90%
 - o. $>90\%$
4. Who performs manual differentials in your laboratory?
 - a. All technologists who perform hematology
 - b. A subset of technologists who perform hematology
5. Does your laboratory use a digital imaging morphology system (eg. Cellavision, Vision Hema, EasyCell assistant, HemeCAM, NextSlide, Cobas m511 etc)?
 - a. Yes
 - b. No
6. Does your laboratory report band neutrophil counts/percentages on manual differentials? (select all that apply)
 - a. No – never (Skip to question #9)
 - b. Yes – always (Skip to question #8)
 - c. Yes, sometimes –per laboratory policy, for specific patient populations and/or clinical scenarios (Proceed to question #7)
 - d. Yes, sometimes - at clinician request (Proceed to question #7)

Supplemental Table 1. Survey questions distributed to the College of American Pathologists program participants (2020-A mailing) (cont.)

7. If your laboratory reports bands for specific patient populations, clinical scenarios and/or at clinician request, which departments send the specimens?
(Select all that apply):
- a. Critical Care (Intensive Care Unit)
 - b. Emergency Department and/or Urgent Care
 - c. General Pediatrics
 - d. Neonatal Intensive Care Unit
 - e. Hematology/Oncology, Adult
 - f. Hematology/Oncology, Pediatric
 - g. Other (please specify): _____
8. Does your laboratory provide a reference range when reporting band counts?
- a. Yes, only for percentage of band counts.
 - b. Yes, only for absolute band counts.
 - c. Yes, for both percentage and absolute band counts.
 - d. No reference range provided
9. What institution type is your laboratory affiliated with (choose one that best describes your practice)?
- a. National or regional reference laboratory
 - b. Community hospital
 - c. University/academic hospital
 - d. Independent local and/or commercial laboratory
 - e. Veteran's hospital
 - f. Other, specify: _____
10. What is your laboratory's monthly volume of all orders for complete blood count (CBC) with differential (include all diffs, automated and manual)?
- a. <100
 - b. 100 – 499
 - c. 500 – 999
 - d. 1,000 – 4,999
 - e. 5,000 – 9,999
 - f. 10,000 – 14,999
 - g. \geq 15,000
11. Where is your laboratory located?
- a. United States
 - b. Canada
 - c. Other

Supplemental Table 2. Morphologic Cell Identification Performance by Laboratories (n = 1,165) for Intended Responses Associated With Survey Responses to 2020-B-VPBS mailing, percent [n = 1180, each for SQ1, SQ2, SQ9, SQ10, SQ11; n = 1165, each for SQ3, SQ4, SQ5, SQ6]

		EASY SEG VDIFF- 05	EASY SEG VDIFF-09	EASY SEG VDIFF-13	EASY BAND VDIFF-02	EASY BAND VDIFF-10	MOD DIFF BAND VDIFF-06	MOD DIFF BAND VDIFF-07	HARD BAND VDIFF-03	HARD BAND VDIFF-11	EASY META VDIFF-04	EASY META VDIFF-08	EASY META VDIFF-12
SQ1	<i>What automated White Blood Cell differentials are reported by instrument?</i>												
	3-part	100.0	100.0	100.0	91.9	97.3	29.7	54.1	0.0	13.5	97.3	64.9	67.6
	5-part	99.6	99.5	99.6	83.0	99.5	28.1	43.2	3.4	11.4	88.5	54.3	56.1
	6-part	99.7	99.3	99.5	80.1	98.6	21.4	36.2	3.1	7.0	87.3	53.1	54.7
SQ2	<i>Does your lab perform manual differentials?</i>												
	NO	100.0	100.0	100.0	93.3	100.0	40.0	60.0	6.7	20.0	93.3	86.7	80.0
	YES	99.7	99.4	99.6	81.7	98.9	24.7	39.8	3.1	9.1	88.1	53.7	55.6
SQ3	<i>What percent of reported differentials are manual differentials</i>												
	<5%	99.1	98.7	100.0	81.0	97.4	32.2	47.1	3.5	10.1	91.2	58.6	57.7
	6-10%	99.7	99.7	99.7	83.7	99.3	22.3	41.7	3.0	8.6	88.1	55.0	58.9
	11-20%	99.7	100.0	99.7	84.9	99.7	21.7	40.6	3.3	10.5	89.2	51.6	48.4
	>21%	100.0	99.0	99.0	76.4	98.7	25.1	32.0	3.0	7.9	83.8	49.5	56.8
SQ4	<i>Who performs manual differentials in your laboratory?</i>												
	All technologists perform hematology	99.6	99.4	99.5	81.7	98.8	24.0	39.7	3.2	9.2	87.6	53.3	55.2
	Hematology technologists	100.0	99.0	100.0	82.6	100.0	33.7	43.2	2.1	9.5	93.7	56.8	61.1
SQ5	<i>Does your laboratory use a digital imaging morphology system?</i>												
	No	99.5	99.4	99.7	83.3	99.1	26.2	42.7	3.7	10.6	88.5	55.3	56.8
	Yes	100.0	99.3	99.3	76.7	98.3	20.4	31.2	1.4	4.6	87.0	48.2	51.6
SQ6	<i>Does your laboratory report band neutrophil counts/percentages on manual differentials?</i>												
	No, never	100.0	99.1	100	86.6	99.1	27.7	51.2	3.3	10.8	91.1	62.4	65.7
	Yes, always	99.8	99.5	99.52	80.4	98.9	23.6	36.9	3.0	9.0	88.2	51.0	52.4
	Yes, sometimes per lab policy or clinical request	98.2	99.1	99.08	82.4	98.2	27.5	40.4	3.7	7.3	81.7	56.5	59.6
SQ9	<i>Which institution type is your laboratory affiliated with (choose one that best describes your practice)?</i>												
	Community	99.9	99.4	99.4	81.8	99.1	25.9	42.1	2.8	8.8	87.3	55.4	57.7
	Independent/ Commercial	98.9	100.0	100.0	90.8	98.9	29.2	50.6	2.3	10.1	94.4	65.2	61.8
	National/ Regional	100.0	100.0	98.6	81.9	100.0	27.8	44.4	1.4	11.1	91.7	50.0	54.2
	Other	98.9	100.0	100.0	87.4	98.9	26.4	50.0	10.2	19.3	88.6	60.2	56.8
	University/ Academic	99.5	98.9	100.0	77.4	97.9	16.6	24.6	1.6	5.4	85.1	43.9	48.7

	Veteran's hospital	100.0	100.0	100.0	72.7	100.0	27.3	45.5	18.2	36.4	100.0	36.4	18.2
SQ10	What is your laboratory's monthly volume of all orders for complete blood cell count with differentials (include all differentials, automated, and manual)?												
	<100-499	99.2	99.6	100.0	84.8	98.7	27.0	46.8	2.9	13.9	91.2	59.7	61.8
	500-4,999	99.8	99.6	99.3	82.4	98.9	25.7	41.6	3.5	8.6	87.9	52.8	54.2
	>5,000	99.8	99.3	99.5	79.4	98.9	22.6	36.0	3.0	8.2	86.3	51.1	53.3
SQ11	Where is your laboratory located?												
	Canada	100.0	99.2	99.2	83.0	98.0	28.7	46.1	2.1	9.8	88.9	59.0	62.7
	Other	98.8	100.0	100.0	88.8	98.8	34.5	45.2	6.0	16.7	96.4	72.6	75.0
	United States	99.6	99.4	99.6	80.5	99.1	23.0	38.2	3.3	8.7	86.8	50.4	51.3



SQ – survey question
 SEG – segmented neutrophil
 MOD – moderately
 DIFF – difficult
 META - metamyelocyte