Letter to the Editor

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A universal reference interval for serum immunoglobulins free light chains may be outdated

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To the Editor,

Serum immunoglobulin free light chain (FLC) measurement has become an important component for diagnosis and management of patients with clonal plasma cell and B-cell proliferative disorders. Quantification of κ and λ FLCs is performed using reagent antibodies that recognize light chain-specific epitopes which are hidden in intact immunoglobulins. An abnormal κ/λ FLC ratio (FLCr) can serve as a serological surrogate for clonality. Although the magnitude of involved to uninvolved FLCr is a factor as one of the myeloma-defining events, the International Myeloma Working Group (IMWG) diagnostic [1] and response [2] criteria also include any abnormal FLCr as potentially significant. Thus, distinguishing normal from abnormal FLC ratios can be clinically important.

The Freelite® assay produced by Binding Site was the first commercial reagent for serum FLC measurement. Reference intervals (RI) for this assay were defined in 2002 using 282 healthy donor serum samples [3]. The authors defined a FLCr "diagnostic range" of 0.26–1.65 based on all 282 samples, as the 5 % false-positive rate that would result from the use of a central 95th percentile RI was considered unacceptable. This specific interval is still cited in the Freelite® assay package insert, and in current IMWG guidelines [1, 2].

Several challenges have been observed with FLC assays, including the impact of poor renal function in decreased clearance and subsequent serum FLC accumulation, suggesting the need for a different RI for renal disease [4–6]. Reagents for FLC measurement are now available from multiple manufacturers and can be performed on different instruments using enzyme immunoassay, nephelometry, or immunoturbidimetry. Freelite® reagents demonstrated an upward drift in κ FLC measurement over time, even when a single platform was used for measurement [7, 8]. Despite these factors, the RI of 0.26–1.65 has not been updated in the IMWG guidelines or package insert.

Here, we report findings from recent proficiency testing (PT) surveys from the College of American Pathologists (CAP). The 2019 SFLC-A 2019 PT survey included one specimen (SFLC-01) with FLCr near the upper limit of the RI. Among the 331 participants who reported a FLCr for this specimen, three results were outliers with values greater than 10 and were excluded from further analysis. The median reported FLCr for this specimen was 1.740. In addition to the quantitative value, laboratories may report a qualitative FLCr interpretation. Among the 253 laboratories that reported a quantitative result and interpretation for the FLCr, 40.3 % reported the qualitative result as "Normal" and 59.7 % reported "Abnormal, High". CAP includes evaluation

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criteria for each analyte in the participant summary and does not utilize a predetermined RI for FLCr interpretation; rather, evaluation criteria are defined as 80 % participant consensus. Thus, for this specimen, the qualitative response was not graded because of inadequate participant consensus.

We plotted each participant's reported quantitative FLCr based on manufacturer and result interpretation (Figure 1). Nonconsensus for FLCr was partially due to the wide distribution of values reported for this specimen, which ranged from 1.09 to 3.70. However, we also observed variation in the cut-off used to designate a FLCr as normal or abnormal, as reflected in the interpretation for results between the highest reported "Normal" value (2.18, dashed line in Figure 1) and the lowest reported "Abnormal, High" value (1.66, solid line in Figure 1). The lowest quantitative result that was reported as "Abnormal, High" was 1.66, consistent with the published RI of 0.26–1.65. However, five participants reported qualitative results greater than 1.65 as "Normal", suggesting that these laboratories use another RI. These five participants used assays/instruments from three different manufacturers.

The Clinical Laboratory Standards Institute (CLSI) document EP28 offers guidelines for determining RIs for quantitative clinical laboratory tests, using as few as 20 samples to confirm and a minimum of 120 to establish a RI. To understand laboratory practices for determining FLCr RI, the CAP Diagnostic Immunology and Flow Cytometry Committee sent supplemental questions in the SFLC-B 2021 PT survey. Participants were asked how quantitative RI for serum FLC and FLCr were established in their laboratories and were able to provide multiple responses. Of the 258 participants that responded to this question, 64.0 % (165 of 258) verified manufacturer-provided RI using 20 or fewer

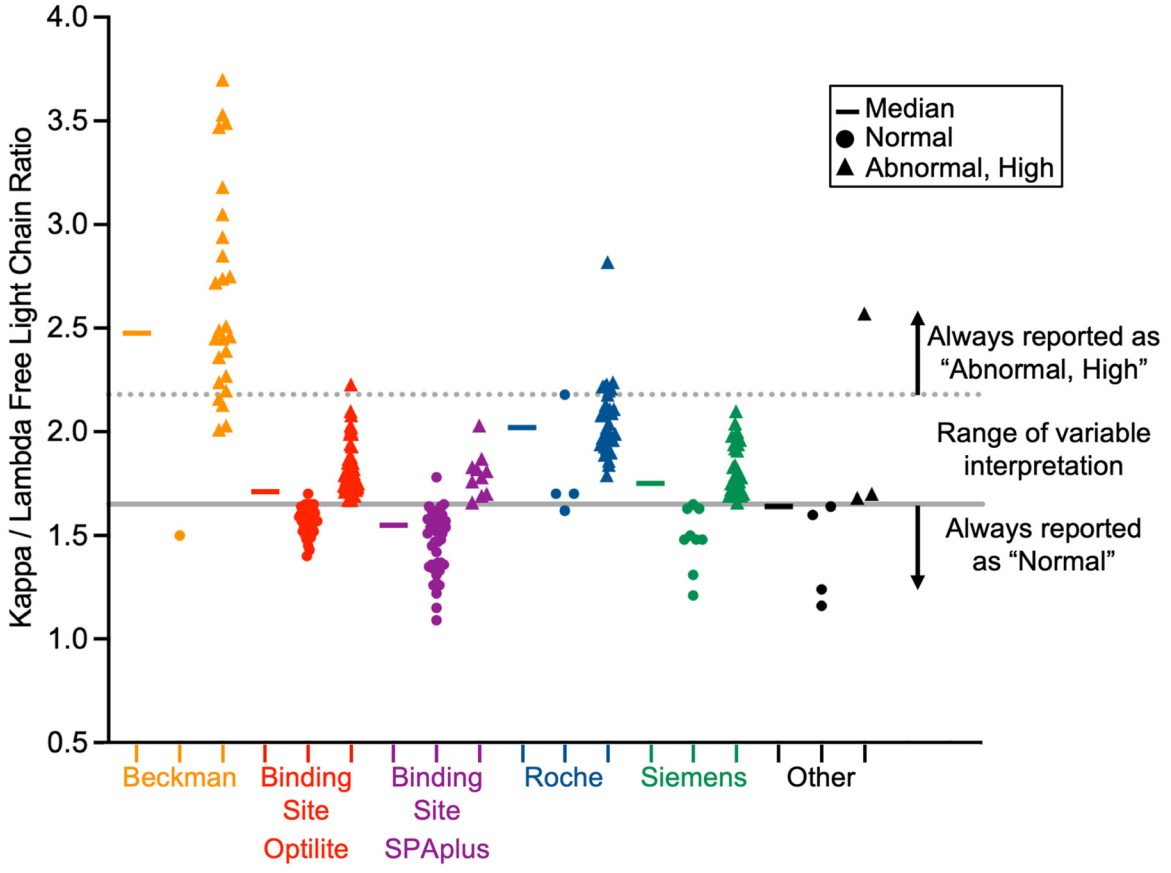

Figure 1: Distribution of serum Kappa/Lambda FLC ratios (FLCr) reported by proficiency testing participants. Individual serum FLCr reported for specimen SFLC-01 by participants in the SFLC-A 2019 proficiency testing survey are shown, grouped and color-coded by reagent and instrument manufacturer. For each manufacturer, values reported as "Normal" are shown as circles in the dot-plot on the left of each manufacturer's section, and values reported as "Abnormal, High" are shown as triangles in the dot-plot on the right. Median values for each reagent and instrument manufacturer are shown as a line. The highest reported "Normal" value (2.18) is indicated by a dashed line; larger values were reported by all participants as "Abnormal, High". The lowest reported "Abnormal, High" value (1.66) is indicated by a solid line; smaller values were reported by all participants as "Normal". Values reported between the dashed and solid lines were variably reported as "Normal" or "Abnormal, High".

specimens and 25.6 % of participants (66 of 258) verified RI using a larger set of specimens (between 21 and 119). A small number of respondents transferred RI from previous assays (9.3 %; 24 of 258), and two laboratories established their RI using 120 or more specimens (0.8 %; 2 of 258). Of the responding laboratories, a large majority (93.8 %) did not change RI since initial verification or establishment. Of the minority of laboratories that did change RI, most reported that the change was a result of a change in manufacturer of reagent or instrument platform used in the laboratory.

Another supplemental question asked for current FLCr RI. Of the laboratories providing an RI, the majority (86.9 %, 192 of 221), reported an interval of 0.26–1.65. Among laboratories that reported other RI, two reported an upper limit of 1.65 with a different lower limit and one reported a lower limit of 0.26 with a different upper limit. Nine laboratories using Diazyme reagents reported RI of 0.22–1.74 (cited in the Diazyme reagent package insert). However, five laboratories using Diazyme reagents reported their interval as 0.26–1.65, suggesting that some laboratories adopt RI used in international guidelines, independent of the laboratory's method. All four laboratories providing RI and using Siemens reagents reported ranges other than 0.26–1.65.

We recognize that individual laboratories may not have sufficient resources to identify, collect, and test a minimum of 120 appropriate samples to independently establish a local FLCr RI. However, we also recognize the concern raised by published studies that the widely used interval of 0.26–1.65 may not be universally appropriate and warrants reconsideration. The use of inappropriate RI may contribute to a high false-positive rate [9] and the use of alternative ranges may reduce false positives, without compromising sensitivity for plasma cell dyscrasias [10]. The latter is especially true for when systemic autoimmune diseases and chronic infections are considered. When a set of 126 reference samples were tested using Freelite® reagents in different immunoturbidimetric and nephelometric platforms, the authors found that the range of 0.26–1.65 did not include all reference samples for any of the four instruments examined [11]. This study established central 95th percentile RI for each instrument, which frequently had higher upper and lower limits than 0.26–1.65.

In light of these CAP survey findings, we support data-driven efforts to establish appropriate RI based on reagent, instrument, and local populations, when possible. We propose that laboratories should not be penalized in external proficiency testing programs if they choose to provide interpretations based on FLCr RI other than 0.26–1.65. As is the case for many analytes, qualitative interpretation for specimens near an interpretive threshold is not likely to achieve consensus among participating laboratories and should not be graded. Finally, we propose that revised clinical guidelines should avoid citing assay or instrument-specific FLCr RI, and that laboratories may consider establishing the FLCr RI using local patient populations in collaboration with clinical teams. Significant further work will be necessary to harmonize clinical serum FLC assays.

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