Reference Interval Transference: Verification Checklist and Flow Chart v.1
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Definitions

Analytical error - is composed of random error (imprecision), measured using coefficient of variation (CV), and systematic error (trueness of measurement), measured using bias.
Coefficient of variation (CV) describes the error around the mean or **standard deviation (SD)** presented as a proportion of the mean; CV = SD/mean.
Histogram - is a graphical representation of the reference data distribution. The quantity (concentration or activity) of the measurand is plotted in intervals on the x-axis and the frequency of measurements within that interval on the y-axis. It is the preferred method for visually presenting reference data and can be used to initially estimate the distribution of data as well as to tentatively identify outliers.
Partitioning criteria - are used to further subdivide a reference population into a more refined demographic. Partitioning creates narrower RI and may be used when there are important biological differences that have an impact on measurable quantities in the partitioned subgroups e.g. age, sex, breed, reproductive status, season, nutrition, etc.
Reference interval (RI) - contains approximately 95% of the possible values between and including an upper and lower limit of a representative, healthy population. Reference interval is preferred over the term reference range.[1]

Candidate RI - is either 1) the RI that may be transferred into the laboratory or 2) the current RI that is verified every 3-5 years.
***De novo* RI** - *de novo* means 'from the beginning', 'afresh', or 'beginning again'. This term refers to RI established by a specific laboratory from reference samples that were collected expressly for this purpose.

Reference Individuals – individual healthy subjects without evidence of disease from the defined demographic that comprise the reference sample group; whose results are used to generate the RI.
Transference - refers to adoption of previously established RI by a laboratory. Procedures for validation/verification of RI must be completed by the adopting laboratory prior to use of the transferred RI to ensure that the RI are appropriate to the laboratory's animal patient population and laboratory methods and quality.
Verification – is used in this document as an equivalent term to validation, which is consistent with the 2008 CLSI documents.[3] It is the process confirming that the use of the candidate RI is acceptable and will not result in clinical error.

Further definitions can be found in the ASVCP Reference Interval Guidelines.
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Checklist

1) Is RI transference indicated? Indications for RI transference or re-verification include but are not limited to:
    a. Replacement of one analyzer with a different analyzer, e.g., new model
    b. Significant changes in:
        i. patient populations
        ii. preanalytical techniques
        iii. analytical quality
    c. Excessive misclassification (false positive and false negative) of results
    d. Periodic reassessment of RI every 3-5 years
2) Does a candidate RI, created in accordance with ASVCP guidelines, currently exist for possible use in the laboratory?
    - If candidate RI are not available, see *de novo* RI generation guidelines
3) Is RI transference likely to be successful? Assess if the following are similar between both the candidate RI and the patient samples in the laboratory verifying the candidate RI:
    a. Processing protocols
    b. Analytical methodology and quality
    c. Quality performance: assessed by determination of SD, CV, and bias and observed total error
    d. Patient population demographics
    - While these items do not have to be identical, verification is more often successful when these factors are closely aligned. If the answer is no (not similar), recommend *de novo* RI generation.)
4) Are the reference values appropriately collected to assess if the RI can be transferred?
    a. Collect samples from 20 reference individuals from the laboratory's healthy patient population that have similar age and sex (or other partitioning factors) distribution as the candidate RI. These should be
        i. Analyzed using the same or similar method that is performing within quality specifications
        ii. Representative of expected inter- and intra-individual variation but free of excessive variation caused by sample storage, handling, etc.
5) Is the distribution of the range of values within the histogram or scatterplot acceptable? See attached examples. As with *de novo* reference intervals, plotting the results in a histogram or scatterplot visually demonstrates the location and distribution relative to the candidate reference intervals as well as highlighting potential outliers.
    a. If the distribution of values roughly covers the range of the candidate RI, verification may be possible
    b. If the distribution of values is visually too narrow or skewed to one region/clustered at the upper or lower extremity versus the candidate RI, it is not acceptable without further investigation.
    - Due to sampling size, occasional reference values may randomly fall to one side of the RI and should be verified with an additional 20 values or a *de novo* RI may be created.
6) Does the appropriate number of reference values fall outside of the candidate RI?
    a. If 1 or 2 of the 20 reference values falls outside of the candidate RI, the RI may be acceptable verification may be possible.
    b. If 3 or more values fall outside of the candidate RI, it is not verified for use. The procedure can be repeated with an additional 20 reference values or a *de novo* RI may be created. The 40 reference values that have been evaluated for transference can be used to establish a *de novo* RI: See *de novo* RI guidelines.

c. If 0 reference values fall outside of the RI, the RI may be too wide and lack sensitivity in detection of nonhealthy individuals. Reassess the distribution (see 5) and consider collection of an additional 20 reference values or a *de novo* RI creation. (See examples).

7) Are there any other clinical or laboratory factors that have not been evaluated and which may be affecting the RI?
   - If there is a question as to clinical relevance, recommend consult with a QA manager or clinical pathologist experienced in RI generation and transference
8) Have all data and results of the transference study been recorded and archived?

Guideline recommendation	**Compliant**	**Guidelines Section**
1) Is RI transference indicated? Indications for RI transference or re-verification include but are not limited to: a) Replacement of one analyzer with a different analyzer, e.g., new model b) Significant changes in: i) patient populations ii) preanalytical techniques iii) analytical quality c) Excessive misclassification (false positive and false negative) results d) Periodic reassessment of RI every 3-5 years	☐ Yes ➔ Proceed to 2. ☐ No ➔ Do not proceed.	Transference Sec 6. P 16
2) Does a candidate RI, created in concordance with ASVCP guidelines, currently exist for possible use in the laboratory?	☐ Yes ➔ Proceed to 3. ☐ No ➔ Generate *de novo* RI.	*De novo* RI P 4-13.
3) Is RI transference likely to be successful? Assess if the following are similar between both the candidate RI and the patient samples in the laboratory verifying the candidate RI: a) Processing protocols b) Analytical methodology and quality c) Quality performance: assessed by determination of SD, CV, and bias and observed total error d) Patient population demographics ➢ While these items do not have to be identical, verification is more often successful when these factors are similar.	☐ Yes ➔ Proceed to 4. ☐ No ➔ Consider *de novo* RI generation.	Transference Sec 1-3. P 14-15
4) Are the reference values appropriately collected to assess if the RI can be transferred? a) Collect samples from 20 reference individuals from the laboratory's healthy patient population that have similar age and sex (or other partitioning factors) distribution as the candidate RI. These should be i) Analyzed using the same or similar method that is performing within quality specifications ii) Representative of expected inter- and intra-individual variation but free of excessive variation caused by sample storage, handling, etc.	☐ Yes ➔ Proceed to 5. ☐ No ➔ Collect appropriate reference values	Transference Sec 4. P 15 & Determination of *de novo* RI Sec 7. P 7.
5. Is the distribution of the range of values within the histogram or scatterplot acceptable? See attached examples. As with *de novo* reference intervals, plotting the results in a histogram or scatterplot visually demonstrates the location and distribution relative to the candidate reference intervals as well as highlighting potential outliers. a) Does the distribution of values roughly cover the range of the candidate RI? b) Is the distribution of values visually too narrow or skewed to one region/clustered at the upper or lower extremity versus the candidate RI?	☐ 5a) Yes ➔ Proceed to 6. ☐ 5b) Yes ➔ repeat 4 w/ 20 additional reference values to confirm transference study or generate *de novo* RI.	Statistical analysis of RI Sec 8.

➢ Due to sampling size, occasional reference values may randomly fall to one side of the RI and should be verified with an additional 20 values or a *de novo* RI may be created.		
6. Does the appropriate number of reference values fall outside of the candidate RI? a. Are 1-2 of 20 reference values outside of the candidate RI? b. Are ≥3 of 20 reference values outside of the candidate RI? i. Too many values out may be variation in sampling or suggest overly narrow candidate RI. c. Are 0 of 20 reference values outside of the candidate RI? ➢ The candidate RI may be appropriate or too wide and lack sensitivity in detection of nonhealthy individuals.	☐ 6a) Yes ➔ Proceed to 7. ☐ 6b) Yes ➔ repeat 4 & 5 w/ 20 additional reference values to confirm transference study or generate *de novo* RI. ☐ 6c) Yes ➔ Repeat 4 & 5 w/ 20 additional reference values to confirm transference study or generate *de novo* RI.	Transference Sec 4. P 15
7. Are there any other clinical or laboratory factors that have not been evaluated and which may be affecting the RI? ➢ If there is a question as to clinical relevance, recommend consult with a QA manager or clinical pathologist experienced in RI generation and transference.	☐ Yes ➔ Proceed to 8 ☐ No ➔ Address factors, repeat 7.	Method verification Sec 4.3, P. 559.
8. Have all data and results of the transference study been recorded and archived?	☐ Yes ➔ Done! ☐ No ➔ record data and results. Repeat 8.	Determination of *de novo* RI Sec 15. P 14.

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