where $\sigma^2_{(1)}$ and $\sigma^2_{(K)}$ are, respectively, the smallest and largest within-lot variance among the K lots. Thus, it is recommended that the current approach of equivalence test for analytical similarity be modified as follows:

1. Randomly select at least two samples from each lot. The replicates will provide independent estimates of within-lot variability $\left(\sigma^2_{WR}\right)$ and lot-to-lot variability $\left(\sigma^2_{BR}\right)$. σ^2_R is the sum of σ^2_{WR} and σ^2_{RB}. In the interest of the same total number of tests, the sponsor can test on two samples from each lot among $K/2$ randomly selected lots.
2. For the establishment of EAC, it is then suggested that $\sigma_{(K)}$ be used to take lot-to-lot and within-lot variabilities into consideration.
3. In the event only one sample from each lot is tested, it is suggested that the upper 95% confidence bound be used as σ_R for establishing EAC for equivalence testing of the identified CQAs in Tier 1. In other words, under the FDA's proposed approach, we will use the following to estimate σ_R:

$$\hat{\sigma}_R = \sqrt{\frac{n-1}{\chi^2_{\alpha/2,n-1}}}\hat{\sigma}_x,$$

where

$\hat{\sigma}_x$ is the sample standard deviation obtained from the n reference lot test values.

$\chi^2_{\alpha/2,n-1}$ is the $(\alpha/2)$th upper quantile of a chi-square distribution with $n-1$ degrees of freedom.

3.6.2 Alternative Approaches

Alternatively, we may consider a Bayesian approach with appropriate choices of priors for the mean and standard deviation of the reference product in order to take into consideration the heterogeneity in mean and variability. The Bayesian approach is to obtain a Bayesian creditable interval which will consider EAC for the assessment of analytical similarity.

3.7 CONCLUDING REMARKS

For purposes of identifying CQAs at various stages of the manufacturing process, most sponsors assign CQAs based on the mechanism of action (MOA) or pharmacokinetics (PK) believed to be relevant to clinical outcomes. It is a reasonable assumption that change in MOA or PK of a given quality attribute is predictive of clinical outcomes. However, the primary assumption that there is a well-established relationship between *in vitro* assays and *in vivo* testing (i.e., *in vitro* assays and *in vivo* testing correlation; IVIVC) needs to be validated. Under the validated IVIVC relationship, the criticality (or risk ranking) can then be assessed based on the degree of the relationship. In practice, however, most sponsors provide clinical rationales