Toxoplasma IgG Quality Control Testing Optimization

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Abstract *- This project explores a lean initiative of a multinational healthcare company related to a Toxoplasma IgG (protozoan parasite) quality control test. The objective was to reduce the test's cycletime, material consumption and labor through an optimization of the testing scheme. A nested study design and a random effects model (also called variance components model) were utilized to assess the test method sources of variability. The test method was used to assess materials at various stages of production by evaluation of assay response and/or sample concentration. Validation runs showed that the test method performs in a manner that is consistent with the variance determined. A new testing scheme was set and its suitability for intended use was demonstrated. As a result, the total annual cost of the test was reduced from $45,736 to $33,320 or by 27 percent (%).*

Toxoplasma Gondii Test

Approximately 85 percent (%) of women of childbearing age in the United States are susceptible to acute infection with the protozoan parasite Toxoplasma gondii. Transmission of T. gondii to the fetus can result in serious health problems, including mental retardation, seizures, blindness, and death. An estimated 400 to 4,000 cases of congenital toxoplasmosis occur in the United States each year.

Serologic tests are used to diagnose acute T. gondii infection in pregnant women. Because false-positive tests occur frequently, serologic diagnosis must be confirmed at a Toxoplasma reference laboratory before treatment with potentially toxic drugs is considered. In many instances, congenital toxoplasmosis can be prevented by educating pregnant women and other women of childbearing age about not ingesting raw or undercooked meat, using measures to avoid cross-contamination of other foods with raw or undercooked meat, and protecting themselves against exposure to cat litter or contaminated soil.

T. gondii is transmitted to humans by three principal routes. First, humans can acquire T. gondii by eating raw or inadequately cooked infected meat, especially pork, mutton, and wild game or uncooked foods that have come in contact with infected meat. Second, humans can inadvertently ingest oocysts that cats have passed in their feces, either from a litter box or from soil (e.g., soil from gardening, on unwashed fruits or vegetables, or in unfiltered water). Third, women can transmit the infection transplacentally to their unborn fetus. In adults, the incubation period for T. gondii infection ranges from 10 to 23 days after the ingestion of undercooked meat and from five to 20 days after the ingestion of oocysts from cat feces [1].

An immunochemical automated analyzer (as shown in Figure 1) is used for quantitative and qualitative measurement of IgG antibodies to toxoplasma gondii in human serum or plasma. This instrument is used in medical laboratories by trained medical personnel. It can process about 100 samples an hour. A multinational healthcare company made this instrument and manufactures the reagents, calibrators and controls need it to process the tests. As part of the manufacture a quality control test is performed in order to determine the optimum sample/control (S/C) ratio of the In-Process Toxo Calibrators B through E.

Figure 1

Immunochemical Automated Analyzer

The testing scheme requires a significant amount of labor, as shown in Table 1. The test consists of a cycletime of 60 hours, a material consumption of $3,600 and a labor activity cost of $2,117 for a total of $5,717 per lot produced. The total annual cost of the test is $45,736, based on an annual production schedule of eight (8) lots. Consequently, the test is considered one of the operations major offenders. Therefore, the sample/control (S/C) test needs to reduce cycletime, material consumption and labor intensive. This lean initiative will focus on reducing the testing scheme of the quality control testing laboratory.

Table 1

Sample/Control (S/C)

Testing Scheme (Current)

	Current		
Code	# Instrument	# Run/Instrument	Total Runs
Calibrator B	4	4	16
Calibrator C	4	4	16
Calibrator D	3	2	6
Calibrator E	2	2	4
			42

Characterization and Confirmation Studies

The lean initiative consists of three steps. During the first step an experiment or characterization study was performed in order to evaluate the test sources of variability. The test method is used to assess materials at various stages of production by evaluation of assay response and/or sample concentration. The test method sources of variability identified were reagent, instrument, runs per instrument and replicates of the sample. A random effects model (also called variance components model) was determined from a nested study design including two (2) reagents lots, five (5) instruments, two (2) runs per instrument, and two (2) samples replicates per run (2 x 5 x 2 x 2) [2]. Refer to Figure 2 for the Nested Study Design (Characterization), Equation (1) for the Variance Components Model and Table 2 for the Variance Components Summary.

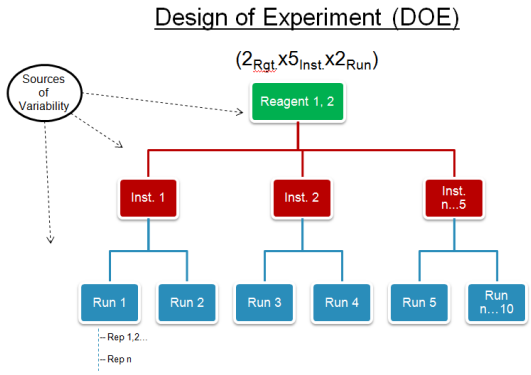

Figure 2

Nested Study Design (Characterization)

$$y_{ij} = \mu + \beta_i + \beta_{j(i)} + \varepsilon_{ij} \qquad (1)$$

Where,

y_{ij} is the response from the *jth* sample nested within the *ith* batch

μ is the mean

β_i is the random effect of the *ith* batch

$\beta_{j(i)}$ is the random effect of the *jth* sample nested within the *ith* batch

ε_{ij} is the residual error

The sum of the variance components of the *jth* sample and *ith* batch provides an estimate of the total data variability (StdDev).

Table 2

Variance Components Summary

Sample	Parameter: Concentration (C) in IU/mL or Response (R) in light	Reagent Variance	Instrument Variance	Run Variance	Replicate Variance	Study Mean
Calibrator A	R	1.01093	1.49177	0.28175	2.59451	212.53
Calibrator B	R	2.03528	2.59859	0.530602	4.45901	189.85
Calibrator C	R	1.02305	0.485548	5.72002	3.96758	164.88
Calibrator D	R	0.108788	2.4973	1.05978	2.31781	110.34
Calibrator E	R	0.346784	0.518535	0.0	2.7299	65.85
Calibrator F	R	0.0	0.830768	0.0	1.50828	35.24
Control Low	C	10.2625	13.2469	51.2304	69.7689	322.87
Control Medium	C	0.0	144.737	94.4959	429.961	833.44
Control High	C	0.0	7332.36	10774.8	15178.6	1998.10

Table 2 shows the Variance Components calculated, where the instrument and replicate factors were identified as the most significant sources of variability. Variance Components were used to calculate the restrictive limits considering the repeatability (within run precision) and intermediate precision (between instruments precision). Table 3 presents the restrictive limits that were determined.

Table 3

Study Restrictive Limits

Samples	Parameter: Concentration (C) in IU/mL or Response (R) in light	Repeatability Acceptance Criteria %CV	Intermediate Precision Acceptance Criteria %CV
Calibrator A	R	≤2.2 %	≤2.1 %
Calibrator B	R	≤3.2 %	≤3.1 %
Calibrator C	R	≤3.5 %	≤3.9 %
Calibrator D	R	≤4.0 %	≤4.3 %
Calibrator E	R	≤7.4 %	≤5.6 %
Calibrator F	R	≤10.4 %	≤8.6 %
Control Low	C	≤7.6 %	≤7.3 %
Control Medium	C	≤7.3 %	≤6.0 %
Control High	C	≤19.1 %	≤19.5 %

$$\%CV = \frac{\text{sample replicate sd}}{\text{sample replicate mean}} \times 100$$

During a second step of the project, a confirmation study (validation) was performed. A nested study design including one (1) reagent lot, two (2) instruments, two (2) runs per instrument, and five (5) samples replicates per run (1 x 2 x 2 x 5) were performed using the nested study design presented in Figure 3. The intended of the confirmation study was to determine if material on test conforms to a predetermine acceptance criteria.

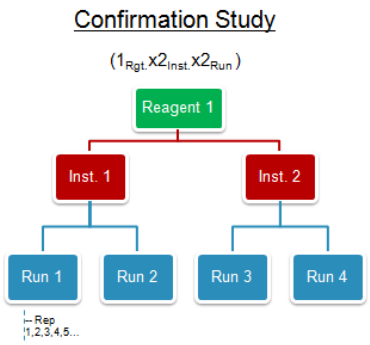

Figure 3

Nested Study Design (Confirmation)

Table 4

Confirmation Study Results

Samples	Parameter: Concentration (C) in IU/mL or Response (R) in light	Repeatability Acceptance Criteria %CV	Results	Intermediate Precision Acceptance Criteria %CV	Results	Acceptance Criteria Met: Yes/No
Calibrator A	R	≤2.2 %	0.8	≤2.1 %	0.8	Yes
Calibrator B	R	≤3.2 %	1.4	≤3.1 %	1.3	Yes
Calibrator C	R	≤3.5 %	0.6	≤3.9 %	2.3	Yes
Calibrator D	R	≤4.0 %	0.9	≤4.3 %	2.4	Yes
Calibrator E	R	≤7.4 %	2.8	≤5.6 %	4.1	Yes
Calibrator F	R	≤10.4 %	6.2	≤8.6 %	3.7	Yes
Control Low	C	≤7.6 %	0.6	≤7.3 %	3.4	Yes
Control Medium	C	≤7.3 %	2.1	≤6.0 %	3.1	Yes
Control High	C	≤19.1 %	5.6	≤19.5 %	6.4	Yes

Table 4 shows that all the acceptance criteria were met. Validation runs showed that the test method performs in the laboratory conforms to the variance determined in the characterization study.

During a third step, a Test Method Application Worksheet (TMAW) that uses the variance components determined in characterization and a new testing scheme were used to demonstrate that the test method is suitable for its intended use. A test method is considered suitable for the intended use if the capability index (Cp) is equal to or greater than 1.0 for in-process testing applications. Refer to Equation (2) for the Cp equation [3].

$$Cp = \frac{USL - LSL}{6\sigma} \qquad (2)$$

USL is the upper specification limit
LSL is the lower specification limit

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n}}$$

σ = standard deviation

$\sum$ = sum of

x = each value in the data set

$\bar{x}$ = mean of all values in the data set

n = number of value in the data set

Table 5 shows that a new testing scheme for in-process test complies with the requirement of a Cp equal to or greater than 1.0.

Table 5

Capability Index (Cp) and Testing Scheme

Parameter	C_P	TM Suitable	LSL	USL	Rgt	Inst per Rgt	Run per Inst	Rep per Run	SD
Calibrator B	1.33	Yes	182	198	1	2	1	27	2.0075
Calibrator C	1.00	Yes	160	170	1	3	4	27	1.6618
Calibrator D	2.37	Yes	100	120	1	2	2	27	1.4046
Calibrator E	1.98	YEs	60	70	1	2	1	27	0.8409

Results

As part of the assessment, the testing scheme was modified, for the process shown in Table 6. The numbers of instruments were reduced for all the calibrators while the numbers of runs per instruments were reduced only for calibrator B and E. Even though, a considerable reduction was reflected on calibrator B, from 16 to 2 total runs. The reduction of the test total runs was 22.

Table 6

Sample/Control (S/C) Testing Scheme

(Current and New)

Testing Scheme (Cycle Time)

Current			
Code	# Instrument	# Run/Instrument	Total Runs
Calibrator B	4	4	16
Calibrator C	4	4	16
Calibrator D	3	2	6
Calibrator E	2	2	4
			42

New			
Code	# Instrument	# Run/Instrument	Total Runs
Calibrator B	2	1	2
Calibrator C	3	4	12
Calibrator D	2	2	4
Calibrator E	2	1	2
			20

Conclusion

The Toxoplasma IgG Quality Control Testing Optimization project was performed in accordance with all the good manufacturing practices in the healthcare industry. No atypical event was observed during the runs. The cycletime, material consumption and labor intensive of the new test were reduced when compared to the previous test, as shown in Table 7. The total annual cost of the test was reduced from $45,736 to $33,320 or by 27%, based on an annual production schedule of eight (8) lots.

Table 7

Total Annual Cost Improvement

QC Testing Scheme

	Current	New	Diff.
Cycletime	3.5 - 4 days (60 hrs)	2 -2.5 days (32 hrs)	1.5 days (28 hrs)
Material Consumption	$ 3,600	$ 3,000	$ 600
Labor Activities Costs	$ 2,117	$ 1,165	$ 952
Total	$ 5,717	$ 4,165	$ 1,552

Total Cost (Annual) = $ 45,736 → **$ 33,320**

References

[1] Jeffrey Jones, M.D., M.P.H., Adriana Lopez, M.H.S., and Marianna Wilson M.S., American Academy of Family Physician, *Congenital Toxoplasmosis*, Retrieved April 2013 from http://www.aafp.org/afp/2003/0515/p2131.html

[2] G. Milliken, D. Johnson, *Analysis of Messy Data Volume 1: Designed Experiments*, Second Edition, Chapman and Hall/CRC, July 26, 2004

[3] NIST/SEMATECH e-Handbook of Statistical Methods, *What is Process Capability?*, Retrieved April 2013 from http://www.itl.nist.gov/div898/handbook/pmc/section1/pmc16.htm