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Evaluation of the CLSI EP26-A protocol for detection of reagent lot-to-lot differences

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ABSTRACT

Background: Verification of new reagent lots is a required laboratory task. The Clinical and Laboratory Standards Institute (CLSI) EP26-A guideline provides a lot-to-lot verification protocol to detect significant changes in test performance. The aim of this study was to compare the performance of EP26-A with our laboratory reagent lot verification protocol.

Methods: Prospective evaluations for two reagent lots each for thyroid stimulating hormone (TSH), thyroglobulin (Tg), thyroxine (T4), triiodothyronine (T3), free triiodothyronine (fT3), and thyroid peroxidase antibody (TPOAb) were performed. The laboratory's lot verification process included evaluation of 20 patient samples with the current and new lots and acceptability based on a predefined criteria. For EP26-A, method imprecision data and critical differences based on previously defined lot-to-lot consistency goals were used to define sample size requirements and rejection limits.

Results: EP26-A required the following number of samples: 23 for TSH, 17 for Tg, 33 for T4, 31 for T3, 48 for fT3, and 1 for TPOAb. Our current protocol and EP26-A were in agreement in 9 of the 12 (75%) paired verifications. Of the 3 discrepant verifications, Tg and TSH reagent lots were rejected by EP26-A due to significant differences at medical decision points; whereas TPOAb was rejected by the current laboratory protocol.

Conclusions: The EP26-A protocol arrived at the same conclusions as our protocol in 75% of the evaluations and required more samples for 4 of the 6 analytes tested. Challenges associated with determining rejection limits and the need for increased sample sizes may be critical factors that limit the utility of EP26-A.

1. Introduction

Clinicians rely on consistent laboratory test results to aid with medical diagnosis, guide treatment plans, or monitor patient responses to therapy. One source of variability in laboratory test results could arise from changes in calibrator and reagent lots [1]. Despite manufacturers using control processes for production of different lots, reagent lot-to-lot differences are often observed and could have a negative impact on patient care if not identified prior to use for clinical testing. Processes to ensure lot-to-lot consistency vary greatly among manufacturers and can be limited due to their inability to test these new lots on patient samples. Considering these challenges and the potential for discrepancies in results due to differences in reagent lots, the verification of new reagent lot performance is a routine, but important laboratory task.

In order to satisfy the College of American Pathologists (CAP) requirements, laboratories must have a protocol for confirming that the new reagent lot compares with the current reagent lot before it is placed into clinical use. However, reagent lot verifications can vary widely among clinical laboratories with regard to the number of samples evaluated, the type of material tested, and the criteria used for acceptance [2]. In 2013, the Clinical and Laboratory Standards Institute (CLSI) published EP26-A, a guideline intended to provide laboratories with a practical protocol for verifying the consistency of the analytical performance of a test between consecutive reagent lot changes [3]. Prior to this publication, there was no standardized protocol or guideline to help laboratories address reagent lot-to-lot verification. The aim of

Abbreviations: AMR, analytical measurement range; CLSI, Clinical and Laboratory Standards Institute; CAP, College of American Pathologists; CD, critical difference; fT3, free triiodothyronine; IRB, Institutional Review Board; S_r, intra-assay imprecision or repeatability; Tg, thyroglobulin; TPOAb, thyroid peroxidase antibody; TSH, thyroid stimulating hormone; T4, thyroxine; S_{WRL}, within-reagent lot imprecision.

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this study was to prospectively compare the performance of EP26-A with our laboratory reagent lot verification protocol for immunoassays.

2. Materials and methods

2.1. Samples and analytes

Samples consisted of residual serum specimens that were clinically tested for the analytes described below using a Beckman Coulter UniCel DxI 800 Immunoassay System (Brea, CA). Samples were selected based on available residual volume and either analyte concentration spanning the measurement range or at preselected clinical decision limits. The following analytes were selected for evaluation: thyroid stimulating hormone (TSH), thyroglobulin (Tg), thyroxine (T4), triiodothyronine (T3), free triiodothyronine (fT3), and thyroid peroxidase antibody (TPOAb). The Mayo Clinic Institutional Review Board (IRB) determined that the study met institutional criteria for a quality assurance/improvement initiative and did not require IRB review.

2.2. Lot–to-lot verification

2.2.1. In-house protocol

Our laboratory's protocol for lot-to-lot verification for immunoassays consists of simultaneously testing 20 patient samples with the current lot and the new lot. Samples are selected to span the analytical measurement range (AMR) of the assay. Lot-to-lot comparison is evaluated using Passing–Bablok regression analysis for estimation of the slope and intercept. Additional parameters evaluated are R^2, individual % difference between paired samples, and mean % difference between the results obtained with the two reagent lots. The acceptance criteria, based on historical goals related to the analytical performance of the assays evaluated, are slope between 0.90 and 1.10, intercept <50% of lowest reportable concentration, $R^2 > 0.95$, and <10% mean difference between reagent lots.

2.2.2. EP26-A protocol

In accordance with the EP26-A protocol, the number of samples required for testing at each target analyte concentration was determined by using the tables provided in the document along with previously defined parameters for inter-assay imprecision (CLSI term within-reagent lot imprecision; S_{WRL}) and intra-assay imprecision (CLSI term repeatability, S_r) [3]. The critical difference (CD) was defined as the maximum difference between the two reagent lots that would be acceptable without having an adverse clinical impact. For this study, the CDs were based on previously established clinical oriented performance goals for lot-to-lot consistency as described [4]. For all assays evaluated, the statistical power was set at 0.80. The number of samples required using these parameters were tested with the current and the evaluation lots. The absolute difference for each sample and the mean difference per target concentration were calculated. The new reagent lot was deemed acceptable if the mean difference per target concentration was less than the predefined rejection limit. For each analyte tested, two new reagents lots were independently compared to the lot in use for clinical testing.

3. Results

3.1. Comparison of sample number requirements for the in-house protocol and EP26-A

For each concentration of analyte tested, the CD was defined to determine the number of samples required for the reagent lot verification. Selected CDs which were based on previously defined lot-to-lot consistency goals [4,5] are shown in Table 1. However, for a few analytes and target concentrations, we found that the target consistency goals could not be used due to limitations of the tables provided in the EP26-A protocol. For example, for total T4 at a concentration of 12.5 mcg/dL, we initially chose a CD of 1.6 based on previously established lot-to-lot consistency goals [4]; the corresponding imprecision at this concentration was 0.81 mcg/dL (S_{WRL}) and 0.60 mcg/dL(S_r). With this information, we were able to locate the desired CD to within-reagent lot imprecision ratio (CD/S_{WRL}). However, we were unable to locate the ratio of repeatability to within-reagent imprecision (S_r/S_{WRL}) that corresponded to the desired CD/S_{WRL} ratio in the table provided in EP26-A. In other similar instances, we took a second approach to determine the lowest possible CDs based on the known S_{WRL} and S_r, while maintaining 80% power for detecting a significant change. Using these two approaches, we were able to determine the number of samples required for each target analyte concentration and the rejection limits for the reagent lot verification (Table 1). In contrast to the 20 samples currently used in our laboratory lot-to-lot verification protocol, evaluation by EP26-A required testing >20 patient samples for 4 of the 6 analytes we evaluated. The number of samples required by EP26-A ranged from 1 sample for TPOAb to 48 samples for fT3.

Table 1
Laboratory parameters used for EP26-A protocol.

Analyte	Target concentration	S_{WRL}	S_r	CD[c]	Target performance goal (percent)[d]	Number of patient samples	Total number of samples
TSH	0.35	0.02	0.01	0.09	25.8[a]	2	23
(mIU/L)	5.4	0.20	0.20	0.24	4.5[a]	21	
Tg (ng/mL)	0.1	0.02	0.01	0.06	N/A	9	17
	2.0	0.08	0.06	0.28	N/A	7	
	10	0.35	0.25	3.2	32[b]	1	
T4	5.0	0.40	0.35	1.0	7[b]	27	33
(mcg/dL)	12.5	0.81	0.60	2.8	12.4[b]	6	
T3 (ng/dL)	80	6.2	8.2	9.5	11.9[b]	10	31
	190	11.8	16.5	11.8	N/A	21	
fT3	2.5	0.20	0.24	0.20	N/A	21	48
(pg/mL)	10.0	1.25	1.11	3.1	N/A	27	
TPOAb	9.0	0.29	0.36	3.3	36.9[b]	1	1
(IU/mL)							

N/A (CD was back-calculated using assay imprecision).
[a] See reference [5].
[b] See reference [4].
[c] Statistical power = 0.8 was used for all analyte concentrations.
[d] CD = target performance goal × target concentration.

3.2. Concordance between the in-house protocol and EP26-A for reagent lot verification

Two new reagent lots for each of the 6 immunoassays were independently evaluated for acceptability according to our current protocol (Table 2) and EP26-A (Table 3). Concordance between the two methods

Table 2
Reagent lot verifications and acceptability using the in-house reagent lot-to-lot verification protocol.

Analyte	Reagent lot	Slope	Y-intercept	R^2	Average % diff. from current lot	Result
TSH	Lot 1	0.97	0.0	0.99	−2.7	Pass
(mIU/L)	Lot 2	0.97	0.0	1.00	−3.7	Pass
Tg (ng/mL)	Lot 1	1.00	0.0	0.99	2.5	Pass
	Lot 2	1.03	0.2	1.00	5.6	Pass
T4	Lot 1	1.01	−0.1	0.99	−1.2	Pass
(mcg/dL)	Lot 2	1.02	−0.1	0.98	0.4	Pass
T3 (ng/dL)	Lot 1	0.94	7.4	0.99	4.4	Pass
	Lot 2	0.96	4.8	0.98	1.1	Pass
fT3	Lot 1	0.96	0.1	1.00	−1.1	Pass
(pg/mL)	Lot 2	0.96	0.1	0.99	0.8	Pass
TPOAb	Lot 1	1.14*	−0.8	0.99	6.3	Fail
(IU/mL)	Lot 2	1.09	−0.3	1.00	3.2	Pass

* Lot deemed unacceptable by protocol.

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