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ORIGINAL ARTICLE

Establishing and evaluating an auto-verification system of thalassemia gene detection results

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Abstract

The manual verification of gene tests is time-consuming and error prone. In this study, we try to explore a high-efficiency, clinically useful auto-verification system for gene detection of thalassemia. A series of verification elements were rooted in the auto-verification system. Consistency check was applied initially as one of the essential elements in our study. One hundred twenty-four archived cases were used to choose the consistency-check rules' indices from routine blood examination and hemoglobin electrophoresis by the receiver operating characteristic curves. Rule 1 and rule 2 established by the chosen indices were compared by their passing rate, consistency with manual validation, and error rate. Finally, 748 cases were used for verifying the system's feasibility by evaluating the passing rate, turn-around time (TAT), and error rate. The rule 2 had a higher passing rate (67.7% vs. 50.8%) and consistency (0.623 vs. 0.364) than the rule 1 with an error rate of zero. In a "live" valuation, the auto-verification system can reduce the TAT and error rate of verification by 51.5% and 0.13%, respectively, with a high passing rate of 82.8%. The auto-verification system for gene detection of thalassemia in this study can shorten the validation time, reduce errors, and enhance efficiency.

Keywords Auto-verification system · Thalassemia · Consistency check · Gene detection

Introduction

Thalassemia, one of the most common monogenic inherited diseases in the world, is caused by the deletion and mutation of one or more globin genes, resulting in inhibition of globin subunit synthesis. In China, thalassemia has high prevalence and variety in the southern regions, such as Guangdong, Guangxi, Guizhou, and Yunnan [1]. In Guangdong province, the overall prevalence of thalassemia carriers is 16.45% [2]. The most common forms are α-thalassemia (12.03%) and β-thalassemia (3.80%) [2], which are classified according to the category of the deleted and mutated globin gene. The α-thalassemia form includes four syndromes—silent carrier (-α/αα), α-thalassemia trait (–/αα or -α/-α), hemoglobin H (HbH) disease (–/-α), and hydrops fetalis (–/–) [3]. The classification of β-thalassemia includes β-thalassemia silent (β^0/β^N or β^+/β^N), β-thalassemia minor (β^0/β^N or β^+/β^N), β-thalassemia intermedia (β^0/β^+ or β^+/β^+), and β-thalassemia major (β^0/β^0, $\beta^0\beta^+$ or β^+/β^+) [3,4]. Clinically, the diagnosis of thalassemia mainly depends on laboratory data that includes routine blood examination (RBE), hemoglobin electrophoresis (HE), and gene analysis. The RBE is characterized by microcytic hypochromic hemolytic anemia, while the HE showed alternations in the quantity of hemoglobins (hemoglobin A, A2 and F) and appearance of abnormal belts such as hemoglobin Bart's and hemoglobins E and S caused by genetic mutations [5]. Gene detection severs as the gold standard for this disease. Though monitoring the whole examination process strictly, laboratory physicians have to evaluate the consistency of thalassemia genotypes identified with the results of RBE and HE in order to prevent delivery of an incorrect result to clinicians when validating the thalassemia genotypes due to the risk of cross contamination and sampling mistake existing in the gene tests. Thus, the process of validation needs spending a lot of time which may prolong the

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laboratory turn-around time (TAT) and requires laboratory; personnel should have high education levels and a full knowledge of the disease to avoid generating post-analytical errors because of subjective and arbitrary reasoning.

To release the clinical laboratory results to the hospital information system (HIS) or clinicians without manual human intervention, auto-verification which can shorten the validation time and reduce the subjective and arbitrary error has been introduced into modern laboratory practice to overcome the drawbacks of manual verification [6,7]. Using predefined computer rules, whereby middleware software resides between the laboratory instruments and the laboratory information system (LIS), auto-verification is recruited to govern the release of laboratory results [8,9]. Although promoting and popularizing auto-verification have been put forward for the last 20 years, the main focus is on the biochemistry, hormone, and hematology tests, whereas little attention is paid on gene tests whose auto-verification rules and parameters are still unclear [7,10,11]. In this study, we employ quality control throughout the whole testing process and consistency check especially in our auto-verification system for gene testing to guarantee the accuracy of the results. Then, we use the TAT, error rate, passing rate, and the External Quality Assessment of the procedures to evaluate the feasibility of the auto-verification rules.

Materials and methods

Methods and instrumentations for gene analysis, routine blood examination, and hemoglobin electrophoresis

Genotypes of α-thalassemia were detected by gap–polymerase chain reaction (Instrumentation: Thermal Cycler, Hema9600, Hema Medical Instrument Co. Ltd. and PowerPac Basic, Bio-Rad Laboratory, Inc.), whereas PCR reverse dot-blot hybridization was applied to the diagnosis of β-thalassemia (Instrumentation: Thermal Cycler, Hema9600, Hema Medical Instrument Co. Ltd. and Hybridization Oven, Yaneng Biotechnology Co. Ltd.) in our clinical laboratory. Routine blood examination was performed with a hematology analyzer (LH 780, Beckman Coulter Inc., CA, USA) based on the theory of electrical impedance. Hemoglobin fractions (HbA, HbA2, HbF, or hemoglobin variants) were evaluated by agarose gel electrophoresis at alkaline pH (Instrumentation: Hydrasys, Sebia Co. Ltd.). All processes were conducted according to the protocol.

Establishment of auto-verification rules

All logic processes and auto-verification rules were established according to Boolean logic and "Design of Algorithms" guidelines in the Auto-10A document issued by the American Clinical Laboratory Standards Institute (CLSI) [11]. Using the middleware software (Data Manager 2 (DM2), Beckman Coulter Inc.) that resides between the laboratory instruments and the LIS [9,12], rules were programmed into the DM2 with the permission to increase and decrease the relevant rules if necessary. The whole elements of the auto-verification rules consisted of the switch of the auto-verification system, samples, instrument alarm, quality controls, thalassemia genotypes, the delta check, and the consistency check, which are illustrated in the flowchart below (Fig. 1). If the result met all the auto-verification rules detailed below, it was classified as the auto-verification result and sent to the HIS directly, if not it was left for manual validation. Cases that failed to be auto-verified were red flagged on the computer screen, which indicated the requirement for a laboratory physician to verify the results. The auto-verification results should be marked differently from the manual-verification ones, which was convenient to identify the auto-verification results when something was wrong.

The switch of the auto-verification system

Critical to the auto-verification system, a switch was programmed to shut down the system compulsively to avoid releasing the wrong results to the clinicians whenever questions of the system emerged. For example, the system would be shut down when it could not verify the results normally. After trouble shooting, the system would be restarted.

Sample rules

The clinical information of the specimens including the patient's name, age, gender, collection time, and type of sample were checked by barcode scanning before analysis. Only qualified samples (the whole blood sample with the correct information of the patient) were permitted to be analyzed in the laboratory. Unqualified samples were redrawn and resent to the laboratory.

Instrument alarm rules

Instrument alarms sounded when errors of reagent, specimen, or apparatus emerged, such as if a reagent was out of date. Once the alarm sounded, the laboratory physician would find out the reasons for this, correct the problems as soon as possible, then restart the process.

Quality control rules

According to the standard operation procedure (SOP) of the laboratory, there were four positive quality control bands in the gap-polymerase chain reaction for α-thalassemia,

including three deletion bands with one normal band. To ensure the success of the test, the appearance of at least one band was necessary. Simultaneously, distilled water was used as a negative control. In the PCR-reverse dot blot hybridization for β-thalassemia, a minimum of six dots had to be colored among the seven normal dots, for all clinical specimens, as part of the quality control. The other two non-conformance terms were as follows: no colored dots on the whole dot blot membrane, and three or more mutation sites with the semaphore. The above quality rules were rooted in the DM2, and the auto-verification system was programmed to alert the investigator when the quality control standards were not met.

Common genotype rule

The genotypic forms of thalassemia are listed in Tables 2 and 3. The three common deletional genotypes of α-thalassemia are defined as the $--^{SEA}$ deletion, the $-\alpha^{3.7}$ deletion and the $-\alpha^{4.2}$ deletion, and the 17 common point mutations associated with β-thalassemia are defined as 41–42 M/N, 654 M/N, −29 M/N, −28 M/N, 71–72 M/N, 17 M/N, 43 M/N, βEM/N, 27/28 M, 31 M/N, −32 M/N, −30 M, 14–15 M, IVS-I-1 M, IVS-I-5 M, IntM, and CAPM [13,14]. All the common genotypes were entered into the DM2 and examined by the system automatically, while the rare types (e.g., δβ-Thalassemia) were left for manual validation.

Age rules

Patients less than 1-year old have their distinct diagnosis criteria for thalassemia [15]. Hence, we specified these kinds of patients for manual verification.

Delta check rules

According to previous studies [11], delta check algorithms should be used to compare the obtained test result with a previous result of the same test obtained over a short period of time from the same patient [16,17] to cut down on potential errors. Due to the uniqueness of the gene, the gene-test results for thalassemia must be in accordance with the history data.

Consistency-check rules

To guarantee the veracity of the auto-verification rules, we initially introduced a consistency check which is referred to

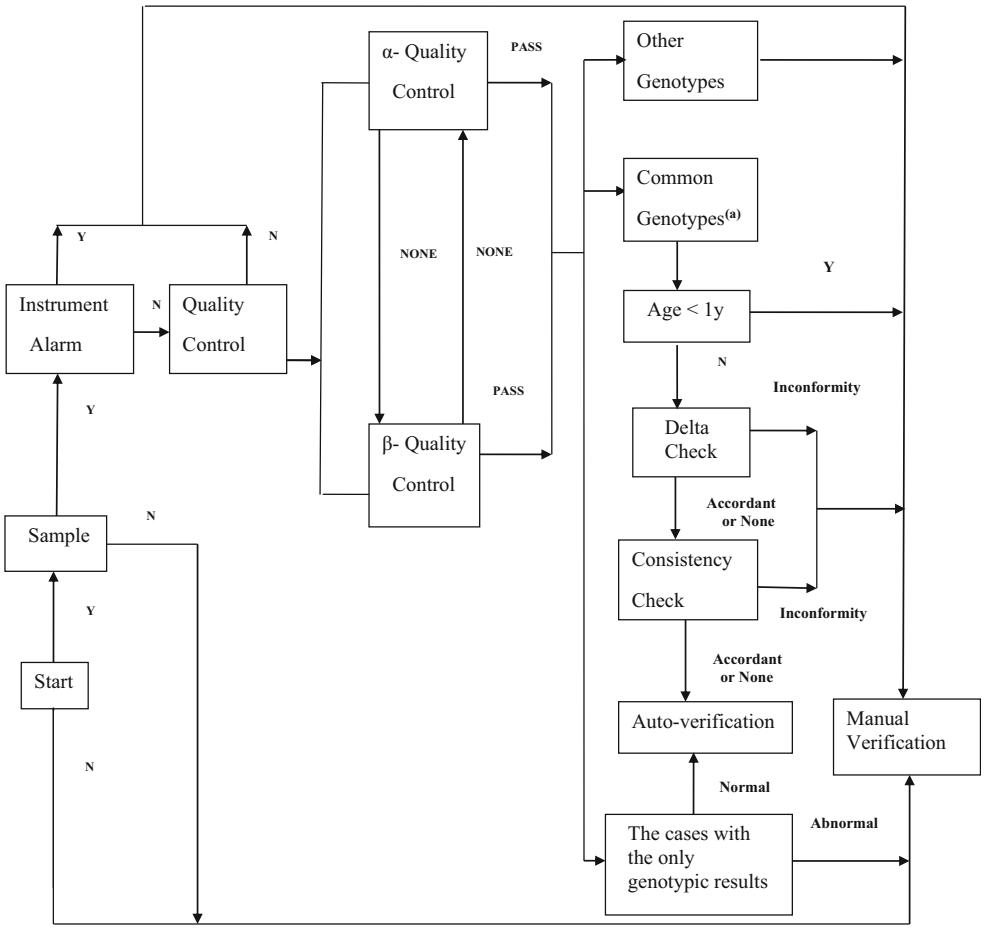

Fig. 1 Diagram of main rules impacting autoverification. **a** The common genotype cases with the other one or two tests

the diseases-related test results of the same patient to detect the accuracy of the results. In our auto-verification system, the consistency check of gene testing was established by consulting in the results of the RBE and HE which was accomplished in two steps [1]: screening indices with the greatest diagnostic performance for thalassemia from RBE and HE [2]. Determining the validation ranges of the indices according to the diagnostic criteria of thalassemia.

Screening indices with the greatest diagnostic performance

To select the best consistency and veracity indices which gene testing results may refer to, a series of alternatives from RBE and HE including red blood cell count (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), adult hemoglobin (HbA), and hemoglobin A2 (HbA2) were compared their diagnostic performance for thalassemia [18] by using the receiver operating characteristic (ROC) curves. One hundred twenty-four archived cases which contained the three necessary laboratory examinations mentioned above were collected for evaluation from January to May 2016. Fetal hemoglobin (HbF) and hemoglobin H (HbH) were directly adopted in the auto-verification system criteria without drawing ROC curves due to their special appearance in some genetypes of thalassemia.

Establishing consistency-check rules with the selected indices

Based on the diagnostic criteria [15] of thalassemia and ROC curve, rule 1 and rule 2 were determined. Then, the passing rate, consistency of manual validation, and error rate were compared between two rules to obtain the optimum one. The Cohen Kappa test was used to evaluate the consistency of the auto-verification and manual validation [6]. The value of the Kappa coefficient represents the degree of consistency, with higher values indicating greater consistency.

Validation of the auto-verification rules

After determining the preferential rules, the entire set of auto-verification rules was programmed into DM2 to validate the feasibility of use and the work efficiency, and the entire validation process was based on the recommendations in the CLSI Auto10-A. Seven hundred forty-eight thalassemia cases from June 2016 to June 2017 were used to evaluate the feasibility of the auto-verification system in a live environment. TAT, passing rate, and error rate were compared between the auto-verification and manual validation methods. After confirmation, the EQA of procedures was taken to monitor the quality of the auto-verification system and general performance levels in different laboratories. Eighty archived cases were selected in 2018 from our medical alliances Haojiang Hospital and Longhu Hospital, respectively, to check the passing rate and error rate of auto-verification.

Statistical analysis

Data were analyzed by the SPSS statistics software package, version 19.0 for Microsoft Windows (SPSS Inc., Chicago, IL, USA). ROC curve was used to choose the best consistency-check indices. The Cohen Kappa test was applied to analyze the consistency of two rules. A $P < 0.05$ was considered significant.

Results

The results of choosing the greatest indices by the ROC curve

According to the areas under the curve (AUC) (Table 1, Fig. 2), MCV (0.852, $P < 0.001$), MCH (0.802, $P < 0.001$), and HbA2 (0.650, $P = 0.017$) for α-thalassemia and MCV (0.834, $P < 0.001$), MCH (0.795, $P < 0.001$), HbA (0.898, $P < 0.001$), and HbA2 (0.853, $P < 0.001$) for β-thalassemia were selected as the indices with the greatest diagnostic performance. As mentioned above, HbF or HbH were also adopted in the auto-verification system, whereas the other types of hemoglobin were left to manual verification.

Results of the establishment and assessment of the rule 1 and the rule 2

By using the ROC curve, the indices including MCV, MCH, HbA, HbA2, HbF, and HbH were determined in order to establish the consistency-check rules. To guarantee efficiency and accuracy, two optional rules were compared. According to the diagnostic criteria of thalassemia, the α-thalassemia silent carrier is characterized by the normal results of RBE and HE with one deletion of genes. So, in rule 1, the unified normal ranges of MCV and MCH were used as the screening conditions for the normal and silent carrier (Table 2). Differently, in rule 2, two ranges of MCV and MCH were used for the normal and silent carrier (Table 3). If the results were in/over the normal ranges, the system would directly go into the next step. While the MCV and/or MCH were below the normal ranges, the serum iron (Fe) was further used to distinguish iron-deficiency anemia from thalassemia.

To identify the better of the two rules, the auto-verification rate, error rate, and consistency of the two auto-verification

Table 1 The results of ROC curve

	α-thalassemia				β-thalassemia			
	AUC	*P*	Sensitivity	Specificity	AUC	*P*	Sensitivity	Specificity
RBC	0.260	< 0.001	0.023	1.000	0.507	0.915	0.467	0.698
HBG	0.646	0.020	0.907	0.465	0.749	< 0.001	0.933	0.581
MCV	0.852	< 0.001	0.977	0.744	0.834	< 0.001	0.833	0.698
MCH	0.802	< 0.001	0.953	0.721	0.795	< 0.001	0.800	0.744
MCHC	0.581	0.194	0.488	0.767	0.564	0.352	0.567	0.628
RDW	0.351	0.062	0.744	0.302	0.286	0.060	0.000	0.000
HbA	0.428	0.252	0.070	1.000	0.898	< 0.001	0.967	0.767
HbA2	0.650	0.017	0.605	0.744	0.853	< 0.001	0.733	1.000

ROC curve the receiver operating characteristic curve, *AUC* the area under the ROC curve, *RBC* red blood cell count, *HGB* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *RDW* red blood cell distribution width, *HbA* adult hemoglobin, *HbA2* hemoglobin A2

rules were each compared with the manual validation (Table 4). The passing rates of rule 1 and rule 2 were 50.8% and 67.7% with the same error rate (0%). The Kappa coefficient between rule 1 (rule 2) and the manual validation was 0.364, $P < 0.001$ (0.623, $P < 0.001$). Thus, rule 2 was selected for the auto-verification system due to its higher auto-verification rate and consistency with an error rate of zero. The final established ranges were listed in Table 3.

Validating of the auto-verification system

By using the auto-verification system for thalassemia in our laboratory over a 10-month period, the number of samples that required manual verification decreased by 82.8% (from 748 to 129, Table 5), and the whole TAT was reduced by 51.5% (from 369.8 min to 179.41 min). The error rate decreased from 0.21 to 0.13%. Results of

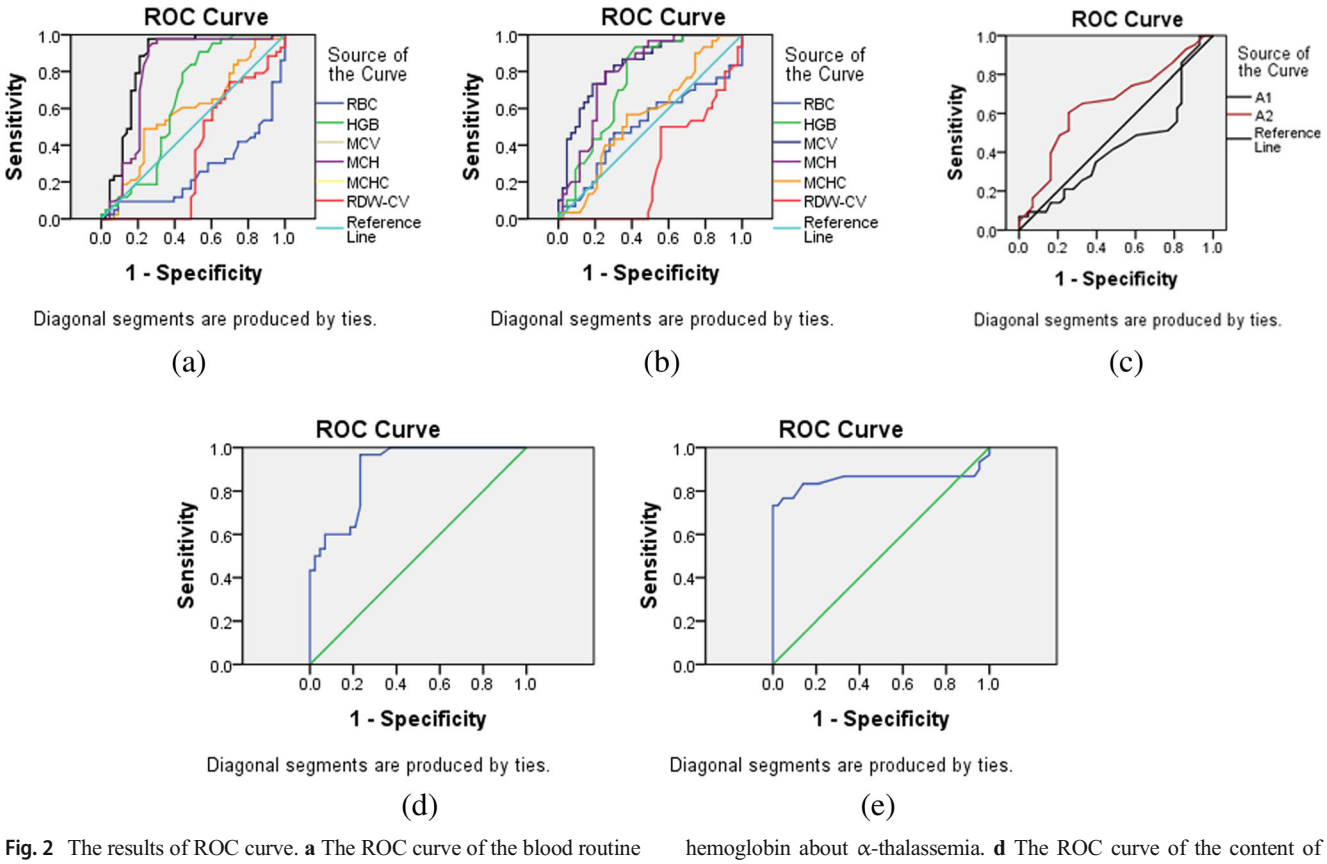

Fig. 2 The results of ROC curve. **a** The ROC curve of the blood routine examination about α-thalassemia. **b** The ROC curve of the blood routine examination about β-thalassemia. **c** The ROC curve of the content of hemoglobin about α-thalassemia. **d** The ROC curve of the content of hemoglobin A about β-thalassemia. **e** The ROC curve of the content of hemoglobin A2 about β-thalassemia

Table 2 The details of rule 1 of the consistency check

		MCV (fl)	MCH (pg)	HbA (%)	HbA2 (%)	HbF (%)	HbH (%)
Normal	αα/αα β^N/β^N						
	αα/αα β^N/β^N	82–100	27–32	≥96.5	2.5–3.5	0–2	0
Silent carrier	-α/αα β^N/β^N	82–100	27–32	≥96.5	2.5–3.5	0–2	0
	-α/αα						
α-Thalassemia trait	--/αα β^N/β^N	< 80	< 27		2.5–3.5/≤2.5	0–2	0
	--/αα						
Hemoglobin H disease	--/-α β^N/β^N	< 80	< 27		≤2.5	0–2	1–40
	--/-α						
Hydrops fetalis	--/--β^N/β^N	< 80	< 27		0	0	0
	--/--						
β-Thalassemia silent	αα/αα β^0/β^N						
	αα/αα β^+/β^N	< 80	< 27	≥96.5	3.2–3.5	0–2	0
	β^0/β^N; β^+/β^N						
β-Thalassemia minor	αα/αα β^0/β^N						
	αα/αα β^+/β^N	< 80	< 27	< 96.5	2.5–3.5/≥3.5	≤5	0
	β^0/β^N; β^+/β^N						
β-Thalassemia intermedia	αα/αα β^+/β^+						
	αα/αα β^0/β^+	< 80	< 27	< 96.5	3.5–4.5	10–50	0
	β^+/β^+						
β-Thalassemia major	αα/αα β^0/β^0						
	αα/αα β^+/β^+						
	αα/αα β^0/β^+	< 80	< 27	< 96.5	≥3.5	> 50	0
	β^0/β^0; β^+/β^+						
	β^0/β^+						
α and β-thalassemia	α and β defect	< 80	< 27	< 96.5	3.2–3.5	/	/

The established ranges of the hematological indices on the basis of the classifications of thalassemia were expounded in detail

MCV mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *HbA* adult hemoglobin, *HbA2* hemoglobin A2, *HbF* fetal hemoglobin, *HbH* hemoglobin H, -$\alpha/\alpha\alpha$ deletions of one α genes, –/$\alpha\alpha$ deletions of two α genes, –/-α deletions of three α genes, --/– deletions of four α genes, β^N β-globin is produced normally, β^0 no β-globin is produced, β^+ some β-globin is produced but less than normal

the External Quality Assessment showed that the auto-verification pass rates of the Haojiang hospital and Longhu hospital were 78.4% and 84.2%, respectively, with the zero error rate.

Discussion

Auto-verification is an essential component for increasing efficiency in clinical laboratories. It is difficult to establish because different laboratory tests require different auto-verification rules, which are based on the clinical values and detection methods. Therefore, it is necessary to set various auto-verification rules according to the laboratory's own reference range, diagnostic criteria, and method. Due to the diversity and laboratory specificity, we only investigated a method and idea of establishing auto-verification rules for laboratory tests in our study, instead of specific rules. The auto-verification system has been used for more than 8 years in our laboratory. We have established the auto-verification rules for quantitative tests (e.g., thyroid function profiles and sex hormone tests, HBV Serological Markers tests and haemostatic function tests) whose quantity control, critical value, gray zone, and limit range were involved in the previous studies. But, in the study, we attempted to explore the auto-verification rules of genetic testing results, which required more attention to avoid random error coming from operation mistakes or sample cross-contamination, instead of critical value, gray zone, and limit range. Therefore, we introduced the consistency check in the auto-verification rules to ensure the accuracy of results.

Although we introduced the consistency check initially, the auto-verification system of genetic detection results also contained the conventional items, such as the samples,

Table 3 The details of rule 2 of the consistency check

		MCV (fl)	MCH (pg)	Fe (μmol/L)	HbA (%)	HbA2 (%)	HbF (%)	HbH (%)
Normal	αα/αα β^N/β^N	< 80	< 27	< 10.7				
	αα/αα β^N/β^N	82–100/>100	27–32/>32		≥ 96.5	2.5–3.5	0–2	0
Silent carrier	-α/αα β^N/β^N	< 80	< 27	< 10.7	≥ 96.5	2.5–3.5	0–2	0
	-α/αα	82–100/>100	27–32/>32					
α-Thalassemia trait	--/αα β^N/β^N	< 80	< 27			2.5–3.5/≤ 2.5	0–2	0
	--/αα							
Hemoglobin H disease	--/-α β^N/β^N	< 80	< 27			≤ 2.5	0–2	1–40
	--/-α							
Hydrops fetalis	--/-- β^N/β^N	< 80	< 27			0	0	0
	--/--							
β-Thalassemia silent	αα/αα β^0/β^N							
	αα/αα β^+/β^N β^0/β^N; β^+/β^N	< 80	< 27		< 96.5	3.2–3.5	0–2	0
β-Thalassemia minor	αα/αα β^0/β^N							
	αα/αα β^+/β^N β^0/β^N; β^+/β^N	< 80	< 27		< 96.5	2.5–3.5/≥ 3.5	≤ 5	0
β-Thalassemia intermedia	αα/αα β^+/β^+							
	αα/αα β^0/β^+ β^+/β^+	< 80	< 27		< 96.5	3.5–4.5	10–50	0
β-Thalassemia major	αα/αα β^0/β^0							
	αα/αα β^+/β^+							
	αα/αα β^0/β^+ β^0/β^0; β^+/β^+ β^0/β^+	< 80	< 27		< 96.5	≥ 3.5	> 50	0
α and β- thalassemia	α and β defect	< 80	< 27		< 96.5	3.2–3.5	/	/

The established ranges of the hematological indices on the basis of the classifications of thalassemia were expounded in detail

MCV mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *HbA* adult hemoglobin, *HbA2* hemoglobin A2, *HbF* fetal hemoglobin, *HbH* hemoglobin H, *Fe* serum iron, -α/αα deletions of one α genes, –/αα deletions of two α genes, –/-α deletions of three α genes, –/– deletions of four α genes, β^N β-globin is produced normally, β^0 no β-globin is produced, β^+ some β-globin is produced but less than normal

instrument alarm, quality controls, and delta check. All of these auto-verification elements are necessary to jointly ensure the veracity of auto-verification. However, in the process of exploring the rules for genetic testing, the most complicated step is the confirmation of consistency-check rules, which have to search the indices with the greatest diagnostic performance and formulate their validation ranges by the diagnostic criteria. In this study, PCR testing for thalassemia was chosen to explore the method about establishing auto-verification rules for genetic detection, because it has a complex, but defined relationship with routine blood examination and hemoglobin electrophoresis.

According to the screening guidelines for thalassemia [15,19–22] characterized by microcytic hypochromic anemia, the disease-related tests must contain RBE and HE, which the laboratory physicians regard as reference indices when they verify the gene results for thalassemia. Other types of anemia and the hemoglobinopathy [5,23] will lead to misdiagnosis when diagnosing thalassemia, which also happens at the stage of consistency checking. So, we need to ensure the accuracy of the indices and their ranges when setting the rules for consistency checking. The indices of RBE and HE perform varying levels of diagnostic performance of thalassemia. This is why we used the ROC curve to evaluate the diagnostic performance of the indices and determined the best ones to be MCV, MCH, HbA, HbA2, and HbF.

When exploring the ranges of the confirmed indices for optimizing our rules, we found that rule 1 showed a lower passing rate and consistency whose cases that did not pass focused on the normal and silent carrier cases. It was because of the disturbance of RBE. As is known, patients with a normal or silent carrier genotype will have MCV and MCH within

Table 4 The comparison of two rules

		Rule 1 No pass	Pass	Rule 2 No pass	Pass	Manual No pass	Pass
Normal	RBE	17	19	2	41	0	43
	HE	10	26	10	32	1	42
	RBE + HE	7	9	1	32	0	43
	Age < 1y	0	43	0	43	0	43
	Genotype	0	43	0	43	0	43
α-Thalassemia	RBE	6[a]	42	0[a]	50	0	51
	HE	4	44	6	44	4	47
	RBE+HE	3	39	1	44	0	51
	Age < 1y	1	50	1	50	0	51
	Genotype	0	51	0	51	0	51
β-Thalassemia	RBE	0	29	0	29	0	30
	HE	9	20	9	20	2	28
	RBE+HE	1	20	1	20	0	30
	Age < 1 year	3	27	3	27	0	30
	Genotype	1	29	1	29	1	29
The Kappa coefficient			0.364 ($P < 0.001$)		0.623 ($P < 0.001$)		
Pass rate (%)			50.8		67.7		82.3
Error rate (%)			0		0		0

RBE the routine blood examination, *HE* the hemoglobin electrophoresis

[a] The difference of the RBE between rule 1 and rule 2 comes from the silent carrier mainly

normal ranges [22,24,25], but could involve other anemia pathogenesis, such as iron deficiency and chronic hemolytic anemia, resulting in an exception changes in the indices. It was also the reason of the poor AUC value of RBC in α-thalassemia. After resetting the rules for MCV and MCH and adding the index of serum iron of the normal and silent carrier

Table 5 The results of verifying the auto-verification rules

	Three Pass A-V	Tests[a] No pass M-V	Two Pass A-V	Tests[b] No pass M-V	One Pass A-V	Tests[c] No pass M-V	Symmation	Auto-verification rate (%)	Manual verification rate (%)	Error rate (%)
Normal	61	37	298	29	41	4	470	85.1	14.9	0.21
Silent carrier	16	4	30	5	6	0	61	85.2	14.8	0
α-Thalassemia trait	48	13	50	4	9	2	126	84.9	15.1	0
HbH disease	6	4	0	0	0	0	10	60	40	0
Hydrops fetalis	0	0	0	0	0	0	0	0	0	0
β-Thalassemia silent	2	0	0	0	0	0	2	100	0	0
β-Thalassemia minor	19	10	23	4	1	1	58	74.1	25.9	0
β-Thalassemia intermedia	0	0	0	0	0	0	0	0	0	0
β-Thalassemia major	0	2	1	0	0	0	3	33.3	66.7	0
α- and β-thalassemia	4	3	4	3	1	3	18	50	50	0
Summation	156	73	405	46	58	10	748	82.8	17.2	0.13
Verification rate (%)	68.1	31.9	89.8	10.2	85.3	14.7				

RBE the routine blood examination, *HE* the hemoglobin electrophoresis, *M-V* manual verification, *A-V* auto-verification

[a] The cases with the results of RBE, HE and PCR

[b] The cases with the results of PCR and RBE or HE

[c] The cases with the only results of PCR

cases in rule 2, the passing rate increased to 67.7% accompanied with a higher consistency and an error rate of zero. Thus, rule 2 is best adapted for the auto-verification system.

To verify the feasibility of this auto-verification system, we evaluated the system over a 12-month period by looking at the TAT, passing rate, and error rate. The manual rate decreased to 17.2% by using the auto-verification system (Table 5), and the TAT was reduced by 51.5%, which freed up the staff to address a subset of difficult results that required careful manual review and scientific research if necessary. At the same time, the error rate decreased from 0.21 to 0.13%. Through application of the auto-verification system, our laboratory decreased the variability associated with manual review, shortened the validation time, and enhanced efficiency. After applying the consistency check, we can reduce the random error by checking the related tests of the same disease, which is identified as an efficient method that can discover test result errors in the shorter time. Moreover, the EQA of our two medical joint hospital showed the good performance of the system.

In our laboratory, the auto-verification system should be permanently in detection mode in case there are network interruptions of the LIS, middleware, and/or the interfaces between these systems. In addition, maintenance of the auto-verification program is required yearly. Despite the advantages of auto-verification, the potential limitations cannot be ignored. First, it is time-consuming to establish, validate, and modify the rules for efficiency and preciseness. Second, unexpected cases that would increase error are not considered, leading to the requirement for continual improvement of the auto-verification rules. Third, the data come from a small sample size, and the implementation time is too short to discover as many problems as possible. Nevertheless, our system provides a feasible method of designing a high-efficiency auto-verification system for genetic testing results and suggests that the perfection of the auto-verification rules requires persistent modification and high attention to detail. In the next phase, we will explore an auto-indicating system for the diagnosis of anemia which can offer a valuable suggestion on the type of anemia or the further laboratory tests to determine etiology on the basis of the same patient's preexisting laboratory tests (e.g., RBE, serum ferritin, knochenbild myelogramm, and so on) in the same time according to the experiences of establishing the auto-verification system of thalassemia gene test results.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics statement This study was performed under the Institutional Review Board approvals from The First Affiliated Hospital of Shantou University Medical College and conducted in accordance with the Declaration of Helsinki. Written informed consents had been obtained from all patients and controls.

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