An Immunoassay for Human Chorionic Gonadotropin in Cerebrospinal Fluid: Validation of a Modified-Approved Method for Accreditation by the College of American Pathologists

Yao Hu[1,*] Jiajin Ni[2,*] Huating Zhang[1] Wenqing Wu[1] Yanwen Chen[1] Ming Guan[1]

[1] Department of Laboratory Medicine, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China.
[2] Department of Nursing, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China.

Address for correspondence Ming Guan, MD, Department of Laboratory Medicine, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, 200040, China (e-mail: guanming@shmu.edu.cn).

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Abstract

Background Human chorionic gonadotropin (hCG) detection in cerebrospinal fluid (CSF) can provide additional value in the diagnosis of germinoma. However, matrix effects can influence the results when alternative sample types are used. Therefore, modified-cleared/approved methods, which are standard methods used outside their intended scope, are of interest. The aim of the present study was to establish a model to validate modified-approved methods in agreement with the College of American Pathologists (CAP) accreditation requirements.

Methods Concentrations of hCG in CSF were determined by means of electrochemiluminescence immunoassay using a Roche Cobas e 602 immunoassay analyzer. Based on the intended use, the following performance characteristics were evaluated: precision, the limit of quantitation (LoQ), and the analytical measurement range (AMR). The reference interval (RI) was also established. For the clinical application study, CSF and serum hCG were measured in 10 patients diagnosed with germinoma.

Results The intra- and inter-assay precisions at two levels (10, 250 IU/L) were 0.64 and 0.57% and 4.26 and 3.54%, respectively. The LoQ for hCG was determined to be 0.25 IU/L. The AMR was set from 0.2 to 1,200 IU/L. The RI for hCG in CSF was below 0.40 IU/L. The CSF hCG levels of 10 patients were all above 0.4 IU/L before therapy.

Conclusion Modified-approved methods were validated and showed that the quality specifications of the medical laboratory have a positive value in the clinical context. The illustration of quantification of hCG in CSF resulted in compliance with the CAP accreditation requirements.

Keywords
- human chorionic gonadotropin
- cerebral spinal fluid
- method validation

[*] Yao Hu and Jiajin Ni contributed equally to this work.

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Introduction

Intracranial germ cell tumors are primary malignant central nervous system (CNS) tumors, which account for 0.5 to 3.0% of pediatric brain tumors in North America and Europe.[1] In Asian countries, the incidence of these tumors is even higher, representing up to 11% of all pediatric CNS tumors.[2] These tumors can be divided into two types, germinomas and nongerminomatous germ cells, both of which have a male preponderance of between 2:1 to 3:1 and tend to arise along the anatomical midline.[1,3] Measurement of serum human chorionic gonadotropin (hCG) is an important adjunct method in the differential diagnosis of these tumors when the lesions contain syncytiotrophoblastic cells, which secrete hCG.[4] Due to the low concentration in serum, the detection of hCG concentrations in cerebrospinal fluid (CSF) is a more robust and sensitive indicator of the presence of tumors and can be a reliable source for guiding treatment and monitoring response to treatment.[5–7]

Currently, all quantitative hCG assays in China are approved by the China Food and Drug Administration (FDA) for application in serum only. Furthermore, it is important to note that matrix effects can influence test results when alternative sample types are used. Therefore, standard methods used outside their intended scope, and validated methods that are subsequently modified, should be validated. The accreditation program of the College of American Pathologists (CAP) states, in the common checklist, that modified FDA-cleared/approved tests or laboratory-developed tests (LDTs) should be validated for the assay before clinical use.

Here, we validated an immunoassay for the detection of hCG in CSF by using a Roche Diagnostics Cobas e 602 analyzer and illustrated a model to validate modified-approved methods in agreement with the CAP accreditation requirement, according to LDTs.

Methods

CSF Samples

The validation study was performed using CSF samples that were sent to the Huashan Hospital Medicine Laboratory for chemistry testing, Shanghai, China, from January 2017 to June 2017. The reasons for physician-ordered lumbar puncture in these patients were fever, headache, or cognitive impairment. All subjects gave informed consent to their surplus CSF samples being used in this study. CSF samples were utilized based upon the following donor criteria: absence of pregnancy, trophoblastic disease or tumor pathology, and no clinical history of blood–brain concentration breakdown or CNS infection. Samples were selected if they were colorless and clear. After donor medical records were reviewed to ascertain CSF quality (protein, glucose, cellular elements, and potentially any other blood–brain abnormalities), a total of 120 surplus CSF samples (61 males, 59 females) from donors between 5 and 81 years old were used for calculating reference intervals (RIs) in the present study.

Analytical Performance

hCG concentrations in CSF were measured using the Elecsys hCG+β quantitative immunoassay from Roche Diagnostics (Cobas e 602; Mannheim, Germany). The following performance characteristics have been evaluated. The limit of quantification (LoQ) was defined as the lowest analyte concentration with a between-series coefficient of variation of 20%. Imprecision, in terms of repeatability and intermediate imprecision, was evaluated. To create samples with two different expected levels (10, 250 IU/L), we diluted high serum hCG samples into the pooled CSF. Serum hCG at a concentration of 1,350.5 IU/L from a patient with nonseminomatous testicular cancer was diluted into pooled CSF. For repeatability, the two concentration samples were assayed 20 consecutive times on the same experimental day. Intermediate imprecision was performed by assaying each sample 20 times in a 20-day period. A recovery test was investigated using the samples mentioned above. The expected dilution concentration for hCG was 10, 125, 250, and 500 IU/L, respectively. Each concentration was assayed in triplicate. The analytical measurement range (AMR), according to the analytical procedure documents of the Clinical and Laboratory Standards Institute EP06-A,[8] was determined by serially diluting serum samples with high hCG concentrations into the pooled patient CSF. The low concentration and high concentration were 0.2 and 1,200 IU/L, respectively. Linearity was analyzed using Microsoft Office Excel 2007 software (Microsoft Corporation, Redmond, Washington, United States). The 95th percentile was used to determine the normal upper limit of CSF hCG RIs with a 95% confidence interval.

Patients

The study involved 10 patients with proven tumorous infiltration of CNS of nonhematologic origin, between 10 and 32 years of age from January 2017 to December 2020. Diagnosis of a germinoma was on the basis of the histopathological findings in three patients or by a combination of clinical factors (including complete response to treatment with chemotherapy and radiotherapy) in another seven patients. From each patient whose medical examination results are anonymously used in this manuscript and the authors have an informed consent for research use at their disposal.

Table 1 Validation of hCG in CSF

Performance characteristics	Obtained results
LoQ	0.20 IU/L
Repeatability	0.64%, 0.57%
Intermediate imprecision	4.26%, 3.54%
Recovery	109–123%
AMR	0.20–1,200.00 IU/L
Reference intervals	$<$ 0.50 IU/L

Abbreviations: AMR, analytical measurement range; CSF, cerebrospinal fluid; hCG, human chorionic gonadotropin; LoQ, the limit of quantification.

Table 2 Imprecision and recovery of hCG in CSF

		Imprecision		Recovery
		Repeatability	Intermediate imprecision	
hCG (IU/L)	Excepted	CV (%)	CV (%)	%
	10	0.64	4.26	123
	125	–	–	118
	250	0.57	3.54	113
	500	–	–	109

Abbreviations: CSF, cerebrospinal fluid; CV: coefficient of variation; hCG, human chorionic gonadotropin.

Table 3 Characteristics of the CSF samples

Age group	Number	Gender (*N*)		CSF hCG level (IU/L)			
		Male	Female	Minimum	Maximum	Median	95th percentile
5–20	25	14	11	< 0.11	0.51	0.24	0.49
21–40	25	12	13	< 0.11	0.55	0.27	0.52
41–60	25	12	13	< 0.11	0.49	0.23	0.45
61–80	45	23	22	< 0.11	0.48	0.22	0.43
Total	120	61	59	< 0.11	0.55	0.25	0.50

Abbreviations: CSF, cerebrospinal fluid; hCG, human chorionic gonadotropin.

Table 4 Demographic and clinical characteristics of the patients

Case	Gender	Age (years)	Clinical symptoms	Serum hCG (IU/L)	Pretherapy CSF hCG (IU/L)	Post therapy CSF hCG (IU/L)	Diagnosis
1	Male	32	Right hemiparesis, headache	< 0.11	1.40	Not done	Nongeminomatous germ cell tumors
2	Male	18	Diabetes insipidus, headache	< 0.11	5.40	Not done	Germinoma
3	Male	14	Diabetes insipidus, headache	4.10	13.10	Not done	Germinoma
4	Female	12	Asthenia, headache	2.50	8.20	Not done	Germinoma
5	Male	25	Right hemiparesis	< 0.11	4.20	Not done	Nongeminomatous germ cell tumors
6	Female	20	Secondary amenorrhea, diabetes insipidus	7.00	20.50	0.22	Germinoma
7	Male	10	Growth arrest, polydipsia, polyuria	5.20	18.10	< 0.11	Germinoma
8	Male	11	Growth arrest, polydipsia, polyuria	3.60	10.10	Not done	Germinoma
9	Male	15	Right hemiparesis, polydipsia, polyuria	10.10	34.00	0.31	Germinoma
10	Male	15	Left hemiparesis, mental change	4.10	14.40	Not done	Germinoma

Abbreviations: CSF, cerebrospinal fluid; hCG, human chorionic gonadotropin.

Statistical Analysis

A range, median, 95th percentile was calculated for continuous variables, and descriptive characteristics of categorical variables were summarized as frequencies. One hundred and twenty samples were divided into four groups based on age, and significant differences were evaluated using one-way analysis of variance (ANOVA) or the Kruskal–Wallis H test. Differences were considered statistically significant at a *p*-value of less than 0.05. Data were analyzed using the SPSS 13.0 (SPSS Inc., Chicago, Illinois, United States).

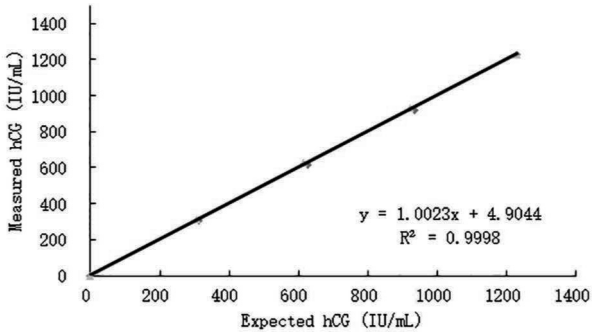

Fig. 1 Linearity of hCG. hCG, human chorionic gonadotropin.

Results

The LoQs for hCG were determined to be 0.25 IU/L. The intra-assay precision was 0.64 and 0.57% and the intermediate imprecision was 4.26 and 3.54% at the concentration 10 and 250 IU/L, respectively. Recovery ranged from 109 to 123%. The dynamic range for hCG assay was set at 0.2 to 1,200 IU/L. The CSF hCG level in 120 samples ranged from < 0.1 U/L to 0.55 U/L. The 95th percentile value was 0.4 IU/L. All results of the validation procedure are reported in ►**Table 1**. The details of both the imprecision and recovery studies are detailed in ►**Table 2**, while linearity is reported in ►**Fig. 1**. Finally, demographic characteristics and hCG concentrations in CSF are presented in ►**Table 3**. Differences in concentrations among the four groups were not statistically significant ($p > 0.05$).

Ten patients were ranging from 10 to 32 years at diagnosis. Eight patients were diagnosed with neurohypophyseal germinoma or intracranial ectopic germinoma on the basis of a satisfactory response to treatment with radiotherapy. Diagnosis of two cases of nongerminomatous germ cell tumors was confirmed by the histopathological findings. Six patients were positive for hCG in both serum and CSF before treatment, but cases 1, 2, 4, and 5 showed elevated hCG only in the CSF. After therapy, there was a marked drop of the CSF hCG level in three patients. The clinical data of all 10 patients are summarized in ►**Table 4**.

Discussion

Indeed, the validation of an immunoassay for hCG in CSF has been previously described,[9,10] but that validation was not performed as per the CAP or any other international accreditation. The CAP checklist provides the criteria needed to validate a laboratory method in terms of performance characteristics and clinical claims. Clinical claims include statements regarding the diagnostic sensitivity and specificity of a test.

To adequately support a claim about the ability to predict the risk of germinoma, we defined the RIs and processed the clinical validation, which included 10 positive clinically diagnosed cases. An established reference value of CSF hCG is useful for detecting the early slight elevation of CSF hCG. When examining the CSF hCG level using the "2 IU/L" upper limit of serum male normal, patient 1 would has been misdiagnosed. With no CSF measurements, patients 1, 2, 4, and 5 would also escape diagnosis because of the low serum hCG concentrations. Repetitive CSF hCG levels in three patients after therapy achieved the results below 0.5 IU/L, which reconfirmed the relevance of the established reference value. Therefore, the CSF hCG assay (intact hormone, free β-fraction, or both) for detecting intracranial germinoma is more sensitive than any other serum parameters. It is a minimally invasive diagnostic method, yet capable enough to ensure crucial information concerning not only the mere presence of intracranial hCG secreting neoplasms but also their closer identification which is fundamental for consecutive therapy and for the patient's prognosis.

The laboratory improvement programs initiated 70 years ago by the CAP, are currently implemented in more than 100 countries, with approximately 8,000 accredited laboratories providing proficiency testing to 20,000 laboratories worldwide.[11] As of 2019, 59 medical laboratories in China have received accreditation from the CAP. The CAP accreditation is a competitive process, which describes specific requirements for the quality and technical aspects of a medical laboratory. The requirements related to the analytical methods used in the candidate laboratories are subject to stringent evaluation, with the main objective to validate analytical methods and to demonstrate their fit-for-purpose.[12] Validation is defined as "where the specified requirements are adequate for the intended use."[13] Nevertheless, the majority of methods are validated by manufacturers; all the procedures, namely laboratory development methods, standard methods used outside their intended scope, and subsequent modified validated methods—usually termed "home-made methods"—need to be validated.

In conclusions, the CAP accreditation requirements concerning the validation of LDTs and modified-cleared/approved methods require a medical laboratory to perform a series of procedures to guarantee that the performance specifications achieved are relevant for the purpose of the test. The CAP accreditation program is a valuable resource that allows researchers to demonstrate the impact of a medical laboratory in a clinical context.

Authors' Contributions

All authors have accepted the responsibility for the entire content of this submitted manuscript and approved submission.

Ethics Approval

This study was approved by the Ethics Committee of Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China (protocol KY-2016–395). All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

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Conflict of Interest

None declared.

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