Utilization of Flow Cytometry for Diagnosis of Hematologic Malignancies in Thailand: Increasing Trends and Diagnostic Yields in 7,982 Samples

Orathai Promsuwicha MSc*, Supattra Kankhao BSc*,
Wayuree Songmuang BSc*, Chirayu U Auewarakul MD, PhD*

** Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

Background: *Diagnosis of hematologic malignancies requires a multidisciplinary approach. Flow cytometry (FCM) has become an essential tool for immunophenotypic studies of malignant hematopoietic cells.*
Objective: *To evaluate the utilization trend of FCM and its diagnostic yields for hematologic malignancy at a major teaching hospital in Thailand.*
Material and Method: *FCM results of bone marrow (BM) and peripheral blood (PB) specimens during 2000-2013 were analyzed and compared to clinical diagnosis.*
Results: *Overall, 7,982 specimens were submitted for diagnostic FCM including 6,561 BM and 1,421 PB. The number of specimens analyzed was 121, 142, 164, 299, 491, 431, 690, 611, 719, 744, 725, 863, 955 and 1,027, respectively, from 2000 to 2013. The most common clinical diagnoses requested for FCM were acute leukemia (5,911 cases, 74%) followed by lymphoma (1,419 cases, 17.8%), and chronic lymphocytic leukemia (CLL) (634 cases, 7.94%). The highest diagnostic yield of FCM was found in acute leukemia cases (69.71%) followed by CLL (35.33%). Only 15.43% of clinically suspected lymphoma cases were positive by FCM. Overutilization of PB (35.6% of cases) instead of BM for lymphoma staging significantly contributed to low diagnostic yields of lymphoma by FCM as circulating tumor cells may not be present in such cases.*
Conclusion: *FCM has an increasing role in the diagnosis of hematologic malignancies in Thai patients over the past 14 years with the highest diagnostic yield in acute leukemia. Appropriate specimen types and study indications are required in order to reduce futility of costly diagnostic tests and improve diagnostic yields.*

Keywords: *Flow cytometry, Diagnostic yields, Acute leukemia, Lymphoma, Hematologic malignancy, Thailand*

J Med Assoc Thai 2014; 97 (12): 1296-301
Full text. e-Journal: *http://www.jmatonline.com*

Hematologic malignancies are a heterogeneous group of disorders associated with high morbidity and mortality rates[1,2]. A multidisciplinary approach is needed to diagnose and classify various types of hematologic malignancies[3,4]. One of the essential tools frequently utilized currently is flow cytometry (FCM) which analyzes and determines the immunophenotypic characteristics of malignant hematopoietic cells[5-8]. The FCM profiles of millions of bone marrow and blood cells can be achieved quickly by flow cytometer. Nevertheless, FCM requires use of costly antibodies to make a definitive diagnosis.

In Thailand, the most common type of hematologic malignancy is malignant lymphoma followed by acute leukemia, chronic myeloid leukemia (CML) and plasma cell myeloma (PCM)[9-14]. The major types of lymphoma in Thailand are diffuse large B-cell lymphoma and peripheral T-cell lymphoma[10-12]. Hodgkin's lymphoma, which is more common in the developed countries, is rare in the Thai population[10,11]. Acute myeloid leukemia (AML) is the most common type of leukemia in Thai adults and acute lymphoblastic leukemia (ALL) is the most common malignancy in Thai children[9,15]. Although CML remains the most prevalent chronic leukemia in Thailand, which remarkably differs from the West, the incidence of chronic lymphocytic leukemia (CLL) appears to be increasing as Thailand enters its aging society era[11,13,16,17].

As FCM has become an integral part in the diagnosis of hematologic malignancies worldwide, this study aimed to analyze retrospectively the trend in the utilization of FCM by clinicians at a major teaching

Correspondence to:
Auewarakul CU, Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Prannok Road, Bangkoknoi, Bangkok 10700, Thailand.
Phone: 0-2419-4449, Fax: 0-2411-2012
E-mail: chirayuaue@yahoo.com

hospital in Bangkok, Thailand. The correlation and discrepancies between clinicians and FCM results were studied to determine the diagnostic yield of FCM for frequently encountered hematologic malignancies in the Thai population.

Material and Method

Patient samples

The present study was a retrospective study approved by the Ethical Committee for Human Research, Faculty of Medicine Siriraj Hospital, Mahidol University. Patient samples from 2000 to 2013 that were sent for FCM were recruited, mainly those with a presumptive clinical diagnosis of acute leukemia, CLL and lymphoma. We recently offered FCM for PCM in 2012; therefore, only 18 cases of PCM were requested in 2012-2013. The clinical diagnosis was retrieved through hospital request forms and compared to FCM results.

FCM analysis

Bone marrow (BM) or peripheral blood (PB) were labeled with the following panel of monoclonal antibodies (BD Biosciences, CA, USA): CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD23, CD33, CD34, CD38, CD41, CD45, CD56, CD57, CD64, CD117, CD79a, CD79b, kappa, lambda, FMC7, cytoplasmic MPO, TdT, and HLA-DR. After 30-minute incubation, cells were lysed in FACS lysing buffer and washed with phosphate-buffered saline (PBS). CD45 and side scatter (SSC) gates were used to select blast windows for multiparameter flow cytometric analysis (FACSCalibur; Becton Dickinson, San Jose, CA, USA.

Statistical analysis

Data were described as median and range when continuous and as absolute and relative frequency when categorical.

Results

There were 7,982 cases submitted for diagnostic FCM including 1,421 PB and 6,561 BM samples. The number of cases sent to the FCM laboratory was 121, 142, 164, 299, 491, 431, 690, 611, 719, 744, 725, 863, 955 and 1,027, respectively, from 2000 to 2013. Fig. 1 shows the trends for acute leukemia, lymphoma, and CLL. Acute leukemia had the most notable increase in the number of FCM requests (8-fold increase in 14 years) whereas in CLL and lymphoma the numbers were much lower. Only 18 cases of PCM were studied in 2012-2013, so the numbers are not displayed in this figure.

Table 1 shows demographic data of specimens submitted to FCM laboratory. The most common clinical diagnoses requested for FCM were acute leukemia (5,911 cases, 74.22%) followed by lymphoma (1,419 cases, 17.82%), and CLL (634 cases, 7.96%). BM samples were more frequently sent to the FCM laboratory than PB (BM 82.2% vs PB 17.8%). More males were found in CLL than females whereas males and females were almost of equal number in acute leukemia and lymphoma. Acute leukemia cases were relatively young (median age, 45) as compared to CLL (median age, 65) and lymphoma (median age, 58).

Table 2 shows the diagnostic yield of each type of hematologic malignancy. The highest diagnostic yield of FCM was found in acute leukemia samples (63.53%) followed by CLL (35.33%). Only 15.43% of lymphoma cases were positive by FCM. A significant proportion of samples sent for lymphoma diagnosis were PB (35.6% of cases), instead of BM, as shown in Table 1.

Among 5,911 clinically suspected acute leukemia samples, 3,755 cases (63.53%) could be confirmed as acute leukemia by FCM (Table 3). Five hundred and twenty-five cases were recognized as acute leukemia in complete remission (CR), 451 cases were diagnosed as myelodysplastic syndrome (MDS), 220 samples as CML, and 74 samples as lymphoma by FCM. If acute leukemia in CR samples are excluded, the diagnostic yield for acute leukemia by FCM became 69.71% out of 5,386 requested cases. Non-diagnostic/ other disorders comprised 15% of clinically suspected acute leukemia requests.

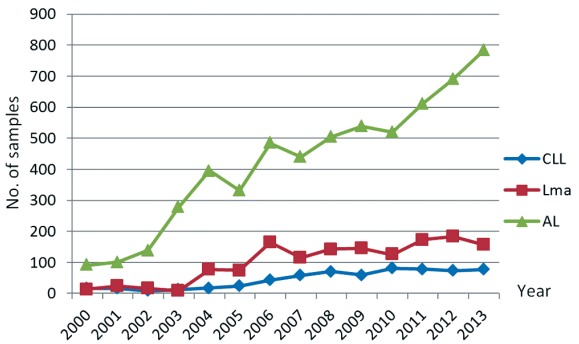

Fig. 1 Increasing number of samples requested for FCM at Siriraj Hospital from 2000-2013 (CLL = chronic lymphocytic leukemia; Lma = lymphoma; AL = acute leukemia).

Table 1. Demographic data of clinically suspected hematologic malignancy *

Clinical diagnosis	n (%)	Gender, n (%)		Age, median (range)	Specimens		Hb (g/dL), median (range)	Hct (%), median (range)	WBC (10^9/L), median (range)	Plt (10^9/L), median (range)
		Male	Female		BM, n (%)	PB, n (%)				
Acute leukemia	5,911 (74.22)	3,001 (50.8)	2,910 (49.2)	45 (12-100)	5,242 (88.7)	669 (11.3)	8.5 (0.7-27)	25.9 (1.56-59.5)	9.30 (0.08-3,768)	56 (0.119-2337)
CLL	634 (7.96)	378 (59.6)	256 (40.4)	65 (21-95)	390 (61.5)	244 (38.5)	10.7 (3.8-16.4)	33.2 (2.5-51.2)	17.45 (0.5-573.69)	150 (3-964)
Lymphoma	1,419 (17.82)	708 (49.9)	711 (50.1)	58 (12-102)	914 (64.4)	505 (35.6)	10.0 (1.1-17.1)	30.7 (2.5-50.7)	6.80 (0.12-5,100)	161 (0.246 -1497)

CLL = chronic lymphocytic leukemia; BM = bone marrow; PB = peripheral blood; Hb = hemoglobin; Hct = hematocrit; WBC = white blood cells; Plt = platelets
* Data retrieved as of 7,982 samples

Table 2. FCM diagnosis of clinically suspected cases of hematologic malignancies

Clinical diagnosis	Total of clinical cases submitted (No. of samples)	Final FCM diagnosis of hematologic malignancies (No. of samples)	Final FCM diagnosis of hematologic malignancies (% of samples)
Acute leukemia*	5,386	3,755	69.71
CLL	634	224	35.33
Lymphoma	1,419	219	15.43

FCM = flow cytometry; CR = complete remission
* Excluding acute leukemia in CR (525 samples)

Discussion

In the present study, the authors described the increasing trend in the utilization of FCM for the diagnosis of adult hematologic malignancies in Thailand from 2000 to 2013. The present study represents the largest study ever reported from Southeast Asia with respect to FCM analysis and diagnostic yields in major hematologic malignancy patients. As acute leukemia is the most prevalent type of leukemia, it is not surprising to have received the most samples from clinicians for acute leukemia diagnosis by FCM. Moreover, FCM is known to be very useful for confirmation and differentiation of various subtypes of acute leukemia according to the immunophenotypic characteristics of leukemic cells[5-8]. The authors found that in a proportion of clinically suspected acute leukemia cases, the final diagnosis had to be changed from AML to ALL and ALL to AML by FCM (data not shown), underscoring the importance of FCM for acute leukemia diagnosis. In addition, not only that acute leukemia subtypes were changed, we also found that many clinically suspected acute leukemia cases turned out to be non-acute leukemia, including MDS (7.62%), CML (3.72%) and CLL/lymphoma (1.25%). In such MDS cases diagnosed by FCM, the criteria to exclude AML was blasts <20%. For clinically suspected acute leukemia cases that turned out to be CLL/lymphoma cases by FCM, myeloid or lymphoid blasts could not be visualized in the blast window to implicate acute leukemia. For clinically suspected acute leukemia cases that turned out to be CML by FCM, we could not significantly find the blasts in the blast window and the predominant granulocytic profile and confirmation by BCR-ABL genetic tests were evident.

With respect to the specimen types, the most common type of received specimen at FCM Laboratory was BM in all categories of hematologic malignancies, and proportionately more for acute leukemia than CLL and lymphoma cases (Table 2). This was not unexpected for acute leukemia as BM aspiration is always indicated in the initial diagnosis of acute leukemia. It was surprising, however, to find that in CLL, Thai clinicians prefer to send more BM than PB (60% and 40%, respectively), and, even more unexpected, was that at least 35% of samples sent for lymphoma diagnosis and staging were PB specimens. Although a minority of lymphoma cases and some subtypes have circulating abnormal cells, BM studies are still needed for diagnosis of stage IV lymphomatous involvement[18,19].

Table 3. FCM diagnosis of clinically suspected acute leukemia samples

Clinical diagnosis, (No. of samples)	FCM diagnosis (No. of cases (%))					
	Acute leukemia	Acute leukemia in CR	MDS	CML	CLL/ lymphoma	Non-diagnostic/ other disorders
5,911	3,755 (63.53)	525 (8.88)	451 (7.62)	220 (3.72)	74 (1.25)	905 (15.31)

MDS = myelodysplastic syndrome; CML = chronic myeloid leukemia

As for the diagnostic yields, 15% of clinically suspected acute leukemia cases could not be confirmed as acute leukemia by FCM (Table 3). These cases included normal BM, normal PB, and other disorders such as thalassemia, aplastic anemia, paroxysmal nocturnal hemoglobinuria, immune thrombocytopenia, immune hemolytic anemia, and other myeloproliferative disorders. Although FCM requested in such settings may be helpful for the exclusion of acute leukemia, the over-utilization of FCM may be a significant healthcare burden in Thailand. The low diagnostic yields of CLL and lymphoma suggested that the morphological diagnosis of CLL and lymphoma by clinicians were inadequate, thus necessitating ancillary FCM analysis[5,18]. On the other hand, inappropriate specimen types sent to FCM, such as PB instead of BM for lymphoma staging, may result in low diagnostic yields by FCM of such cases[20]. The authors have previously reported that the correlation between BM FCM and BM biopsy pathology was better than BM aspirate morphology and BM biopsy pathology for marrow lymphomatous involvement[19]. Risk factors for BM involvement should be taken into consideration when BM staging is performed[21].

In conclusion, the present study reveals an increasing trend of FCM submission requests for hematologic malignancy diagnosis in Thai patients, particularly for acute leukemia. This trend represents a useful estimate for the development of FCM's efficient support strategies for the increasing numbers of such samples in the future. A significant proportion of clinically suspected hematologic malignancy cases could not be confirmed as such by FCM. Overutilization of PB instead of BM for lymphoma staging contributed to the lowest diagnostic yield of lymphoma by FCM. Appropriate study indications and specimen types for FCM analysis should thus be required in order to reduce the futility of costly diagnostic tests and improve diagnostic yields.

What is already known on this topic?

This study is the first report of the trends of flow cytometry in hematologic malignancy in Thailand and the largest series ever reported.

What this study adds?

This study shows the trend of FCM submission requests for hematologic malignancy diagnosis in Thai patients. This trend represents a useful estimate for the development of FCM's efficient support strategies for the increasing numbers of such samples in the future.

Acknowledgment

The authors wish to thank the staffs of the Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, for the excellent care of the patients in the study. CUA is the current recipient of Siriraj Chalermprakiate Fund, Faculty of Medicine Siriraj Hospital and the former recipient of Mahidol University Research Grant.

Potential conflicts of interest

None.

References

1. Storm HH, Klint A, Tryggvadóttir L, Gislum M, Engholm G, Bray F, et al. Trends in the survival of patients diagnosed with malignant neoplasms of lymphoid, haematopoietic, and related tissue in the Nordic countries 1964-2003 followed up to the end of 2006. Acta Oncol 2010; 49: 694-712.
2. Horster S, Stemmler HJ, Mandel PC, Muck A, Tischer J, Hausmann A, et al. Mortality of patients with hematological malignancy after admission to the intensive care unit. Onkologie 2012; 35: 556-61.
3. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009; 114: 937-51.
4. Jaffe ES. The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. Hematology Am Soc Hematol Educ Program 2009; 523-31.
5. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood

2008; 111: 3941-67.
6. Goolsby CL, Paniagua M, Marszalek L. Clinical flow cytometry: a transition in utilization. Cancer Treat Res 2004; 121: 239-57.
7. Wongprajun S, Auewarakul CU. A method comparison study of flow cytometry and cytomorphology to determine the percentages of blasts in patients with acute leukemia after induction and consolidation chemotherapy. J Med Assoc Thai 2010; 93 (Suppl 1): S157-64.
8. Udomsakdi-Auewarakul C, Promsuwicha O, Tocharoentanaphol C, Munhketvit C, Pattanapanyasat K, Issaragrisil S. Immunophenotypes and outcome of Philadelphia chromosome-positive and -negative Thai adult acute lymphoblastic leukemia. Int J Hematol 2003; 78: 337-43.
9. Greenberg PL, Gordeuk V, Issaragrisil S, Siritanaratkul N, Fucharoen S, Ribeiro RC. Major hematologic diseases in the developing world-new aspects of diagnosis and management of thalassemia, malarial anemia, and acute leukemia. Hematology Am Soc Hematol Educ Program 2001; 479-98.
10. Intragumtornchai T, Wannakrairoj P, Chaimongkol B, Bhoopat L, Lekhakula A, Thamprasit T, et al. Non-Hodgkin's lymphomas in Thailand. A retrospective pathologic and clinical analysis of 1391 cases. Cancer 1996; 78: 1813-9.
11. Sukpanichnant S. Analysis of 1983 cases of malignant lymphoma in Thailand according to the World Health Organization classification. Hum Pathol 2004; 35: 224-30.
12. Rattarittamrong E, Norasetthada L, Tantiworawit A, Chai-Adisaksopha C, Nawarawong W. CHOEP-21 chemotherapy for newly diagnosed nodal peripheral T-cell lymphomas (PTCLs) in Maharaj Nakorn Chiang Mai Hospital. J Med Assoc Thai 2013; 96: 1416-22.
13. Au WY, Caguioa PB, Chuah C, Hsu SC, Jootar S, Kim DW, et al. Chronic myeloid leukemia in Asia. Int J Hematol 2009; 89: 14-23.
14. Niparuck P, Sorakhunpipitkul L, Atichartakarn V, Chuncharunee S, Ungkanont A, Aungchaisuksiri P, et al. Treatment outcome of thalidomide based regimens in newly diagnosed and relapsed/refractory non-transplant multiple myeloma patients: a single center experience from Thailand. J Hematol Oncol 2010; 3: 1.
15. Yeoh AE, Tan D, Li CK, Hori H, Tse E, Pui CH. Management of adult and paediatric acute lymphoblastic leukaemia in Asia: resource-stratified guidelines from the Asian Oncology Summit 2013. Lancet Oncol 2013; 14: e508-23.
16. Sriphatphiriyakun T, Auewarakul CU. Clinical presentation and outcome of Thai patients with chronic lymphocytic leukemia: retrospective analysis of 184 cases. Asian Pac J Allergy Immunol 2005; 23: 197-203.
17. Promsuwicha O, Songmuang W, Auewarakul CU. Utilization of a scoring system for diagnosis of chronic lymphocytic leukemia in Thai patients. J Med Assoc Thai 2011; 94 (Suppl 1): S232-8.
18. Talaulikar D, Dahlstrom JE. Staging bone marrow in diffuse large B-cell lymphoma: the role of ancillary investigations. Pathology 2009; 41: 214-22.
19. Promsuwicha O, Songmuang W, Auewarakul CU. Detection of lymphoid neoplasms in bone marrow by flow cytometric analysis and comparison to bone marrow aspiration and biopsy. J Med Assoc Thai 2013; 96 (Suppl 2): S210-5.
20. Davis BH, Holden JT, Bene MC, Borowitz MJ, Braylan RC, Cornfield D, et al. 2006 Bethesda International Consensus recommendations on the flow cytometric immunophenotypic analysis of hematolymphoid neoplasia: medical indications. Cytometry B Clin Cytom 2007; 72 Suppl 1: S5-13.
21. Kittivorapart J, Chinthammitr Y. Incidence and risk factors of bone marrow involvement by non-Hodgkin lymphoma. J Med Assoc Thai 2011; 94 (Suppl 1): S239-45.

การใช้โฟลซัยโตเมทรีเพื่อตรวจวินิจฉัยมะเร็งระบบโลหิตในประเทศไทย: แนวโน้มการส่งตรวจที่เพิ่มขึ้นและผลการวินิจฉัยในสิ่งส่งตรวจ 7,982 ตัวอย่าง

อรทัย พรหมสุวิชา, สุพัตรา ก้านขาว, วยุรี สองเมือง, จิรายุ เอื้อวรากุล

ภูมิหลัง: *การวินิจฉัยมะเร็งระบบโลหิตต้องอาศัยวิธีการตรวจหลากหลายวิธี โฟลซัยโตเมทรีเป็นอีกวิธีหนึ่งที่มีความจำเป็นสำหรับการศึกษาลักษณะทางอิมมูโนฟีโนทัยป์ของเซลล์มะเร็ง*

วัตถุประสงค์: *เพื่อประเมินแนวโน้มของการส่งตรวจด้วยโฟลซัยโตเมทรีและผลการวินิจฉัยมะเร็งระบบโลหิตด้วยโฟลซัยโตเมทรี ณ คณะแพทยศาสตร์ ศิริราชพยาบาล*

วัสดุและวิธีการ: *ทำการศึกษาข้อมูลย้อนหลังทั้งจากไขกระดูกและเลือด ตั้งแต่ พ.ศ. 2543 ถึง พ.ศ. 2556 โดยเปรียบเทียบผลของการตรวจด้วยโฟลซัยโตเมทรีกับการตรวจวินิจฉัยทางคลินิก*

ผลการศึกษา: *โดยรวมมีผู้ป่วยจำนวนทั้งสิ้น 7,982 ราย ที่มีการส่งตรวจด้วยวิธีโฟลซัยโตเมทรี เป็นการส่งตรวจไขกระดูก 6,561 ราย และส่งเลือด 1,421 ราย โดยมีจำนวนที่ส่งตรวจสำหรับ พ.ศ. 2543 ถึง พ.ศ. 2556 เรียงตามลำดับปี ดังนี้ 121, 142, 164, 299, 491, 431, 690, 611, 719, 744, 725, 863, 955 และ 1,027 ราย โรคที่มีการส่งตรวจโฟลซัยโตเมทรีมากที่สุดคือ มะเร็งเม็ดเลือดขาวชนิดเฉียบพลัน (5,911 ราย คิดเป็นร้อยละ 74) ตามมาด้วยมะเร็งต่อมน้ำหลือง (1,419 ราย คิดเป็นร้อยละ 17.8) และมะเร็งเม็ดเลือดขาวลิมฟอยด์ชนิดเรื้อรัง (634 ราย คิดเป็นร้อยละ 7.94) ผลการวินิจฉัยทางคลินิกที่ได้รับการยืนยันด้วยโฟลซัยโตเมทรีในอัตราสูงที่สุดคือ มะเร็งเม็ดเลือดขาวชนิดเฉียบพลัน (ร้อยละ 69.71) ตามมาด้วยมะเร็งเม็ดเลือดขาวลิมฟอยด์ชนิดเรื้อรัง (ร้อยละ 35.33) โดยมะเร็งต่อมน้ำหลืองได้รับการยืนยันด้วยโฟลซัยโตเมทรีเพียงร้อยละ 15.43 ของจำนวนที่ส่งตรวจทั้งหมด การส่งตรวจมะเร็งต่อมน้ำหลืองโดยใช้เลือด (ร้อยละ 35.6 ของจำนวนทั้งหมด) แทนที่จะส่งไขกระดูก อาจเป็นเหตุผลหนึ่งที่ทำให้วิธีโฟลซัยโตเมทรีสามารถยืนยันการวินิจฉัยมะเร็งต่อมน้ำเหลืองได้น้อย เนื่องจากผู้ป่วยอาจไม่มีเซลล์มะเร็งในกระแสเลือด*

สรุป: *การศึกษานี้แสดงให้เห็นว่ามีแนวโน้มการส่งตรวจมะเร็งระบบโลหิตด้วยวิธีโฟลซัยโตเมทรีมากขึ้นอย่างชัดเจนในระยะเวลา 14 ปีที่ผ่านมา โดยเฉพาะอย่างยิ่งในมะเร็งเม็ดเลือดขาวเฉียบพลัน อย่างไรก็ตาม ชนิดของตัวอย่างส่งตรวจและข้อบ่งชี้ที่เหมาะสมทางคลินิกจะช่วยลดการสิ้นเปลืองของการตรวจวินิจฉัยราคาแพง และเพิ่มผลการตรวจวินิจฉัยให้มีประสิทธิภาพดียิ่งขึ้น*