# The Efficiency and Cost Effectiveness of Diagnostic Tests for Infectious Mononucleosis

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The problem of diagnosis and appropriate treatment of patients presenting with pharyngitis is a common occurrence in family practice. The study of these patients includes laboratory tests to differentiate between infectious mononucleosis and other bacterial and viral infections.

This study reviews the diagnosis of infectious mononucleosis in two large ambulatory populations, where different approaches were used. In one approach, all laboratory tests were concurrent, while in the other, serology was performed only after satisfaction of hematologic criteria for infectious mononucleosis. In the latter case, sequential use of laboratory tests resulted in a significant improvement in cost effectiveness. In both approaches, no appreciable gain was obtained from heterophil titers. Since the heterophil titer in confirmed cases of infectious mononucleosis does not correlate with prognosis or severity of the disease, this procedure can be replaced by the Monospot/"monoscreen" test alone.

Pharyngitis with fever, adenopathy, and fatigue is commonly encountered in everyday practice, often raising the question of infectious mononucleosis. Concern over the degree of accuracy of diagnosing infectious mononucleosis raises the issue of cost effectiveness of relevant laboratory procedures and, consequently, cost-benefit to the patient.

The sera of patients with infectious mononucleosis contain antibodies against sheep erythrocytes in concentrations far above normal.[1] Infection by the Epstein-Barr virus is responsible for this disease and presumably responsible for the heterophil reaction.[2-7] The heterophil antibody associated with infectious mononucleosis is specific,[8] while the Forssman heterophil antibody reacts with antigens unrelated to those stimulating their production.[9] A differential heterophil agglutination test[10] (Paul-Bunnell-Davidsohn test) has been widely accepted as a valuable means of diagnosing infectious mononucleosis. A modification of this test[11] (heterophil specific for infectious mononucleosis) using horse erythrocytes* was used at the University of Washington Hospital to differentiate infectious mononucleosis from syndromes with similar symptomatology. At the University of Washington student Hall Health Center, an initial screening based on the number of abnormal lymphocytes was employed to more selectively use agglutination tests. This study is an analysis of the relative efficiency and cost effectiveness of these two approaches in identifying patients with serologic criteria of infectious mononucleosis.

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*Marketed as the Monospot test by Ortho Diagnostics, Inc.

**Table 1. A Comparison of Two Diagnostic Methods for Infectious Mononucleosis**

| Diagnostic Method | University Hospital Method I | Hall Health Center Method II |
|---|---|---|
| Selection of patients | Clinical grounds | Clinical and hematologic grounds |
| Serological testing | Monospot concurrent with WBC, differential, and smear | 'Monoscreen' conditional to satisfaction of clinical and hematologic criteria |
| Confirmatory serology | Differential heterophil conditional to a positive Monospot | Heterophil following positive 'monoscreen' |

## Materials and Methods

Two patient populations were surveyed. The first consisted of 1,712 patients between 14 and 30 years of age, seen over a $3^1/_2$ year period at the University Hospital, Harborview Medical Center, and other affiliated hospitals in Seattle, Washington. The initial test used in patients suspected of infectious mononucleosis in this group was the Ortho Monospot test, a macro agglutination slide test (heterophil specific for infectious mononucleosis) carried out at the University of Washington Immunology Laboratory. Criteria for use of the Monospot test were based entirely on clinical grounds such as pharyngitis, fever, adenopathy, fatigue, and occasionally splenomegaly. Whenever a positive Monospot agglutination occurred, a presumptive heterophil was titered, using sheep erythrocyte reagent in the absence of complement (complement was inactivated by heating to 56.0 C for 30 minutes). Differential absorptions with guinea pig kidney antigen (to remove Forssman antibodies) and beef erythrocytes (to remove the antisheep agglutinins in infectious mononucleosis) were performed. A presumptive titer of 1:224 was considered diagnostic of infectious mononucleosis if the guinea pig absorption titer was decreased fourfold or less. A titer of 1:16 or less in the beef cell absorption test was considered confirmatory.[10,11]

The second population consisted of 1,969 university students, aged 18 to 25 years, seen at the Hall Health Center, University of Washington, Seattle, Washington, over a four-year period. The tests on these students were performed at the Hall Health Laboratory and followed a different format. The first screening procedure for any student with a sore throat and lymphadenopathy consists of an initial leukocyte count and differential as well as a blood smear. Serologic testing with the "monoscreen" was performed only when the following criteria were met: an absolute increase in mononuclear cells in the blood greater than 4,000/cu mm or a relative increase of greater than 50 percent, with atypical lymphocytes of at least 15 percent of the total leukocyte count.[12-14] The "monoscreen" test consisted of the use of a drop of 25 percent suspension of washed sheep cells added to a drop of the patient's serum on a glass slide. The two drops were mixed with an applicator stick exactly ten stirring motions, tilted back and forth five times, and immediately checked for macro-agglutination under a light source against a white background. If there was positive agglutination, a heterophil titer was next performed, using washed sheep erythrocytes with patient's serum in which complement had been inactivated at 56.0 C for 30 minutes, as in the presumptive heterophil test at the University of Washington Immunology Laboratory. However, no absorptions with guinea pig antigens were performed to differentiate the heterophil of infectious mononucleosis from the Forssman antibody. Table 1 illustrates the two methods of diagnosis.

## Results

Of the 1,794 tests performed at the University Hospital (Method I), 104 patients had a positive Monospot test. Ninety-seven of these patients were definitively diagnosed as having infectious

**Table 2. Efficiency of Diagnosis**

| Method I | Number of Patients | Method II | Number of Patients |
|---|---|---|---|
| Selected on clinical grounds | 1,712 | Selected on clinical and hematological grounds | 1,969 |
| Monospot positive | 104 | 'Monoscreen' positive | 558 |
| Differential heterophil positive | 97 | Heterophil positive | 553 |
| % positive | 5.6 | % positive | 28.1 |

mononucleosis by the differential heterophil titers. Five patients had positive Monospot tests twice. One patient had the positive Monospot test repeated five times, and another patient had the positive test repeated 11 times. The incidence of positive diagnosis by the Monospot test was 6.1 percent. Of the 1,794 Monospot tests performed, seven were considered positive but had titers below the diagnostic level by differential agglutination. On subsequent testing, they became negative by Monospot test, or when positive, titers remained below the diagnostic level by differential agglutination. Out of the 104 positive Monospot tests, therefore, seven could be considered as falsely positive, giving an incidence of 6.7 percent. Among the 1,688 negative tests, there were 73 patients who had a second negative test and six patients who had three negative tests.

Of the 1,969 tests performed at the Hall Health Center, where an initial leukocyte count, differential, and blood smear were performed as a first screen before the "monoscreen" (Method II), 558 patients were found to be positive by "monoscreen" (28.3 percent). Of these patients, 553 were diagnosed as having infectious mononucleosis by heterophil agglutination test. The incidence of positive diagnosis in this group of patients was 28.1 percent. The efficiency of these two approaches to diagnosis is summarized in Table 2.

The calculation of cost effectiveness is based on WBC, differential, and blood smear; Monospot/"monoscreen;" and heterophil titering costs. The average charge in the community is $10 for WBC, differential, and blood smear, $6.50 for Monospot or "monoscreen," and $6.50 for heterophil titering, or $23 for hematologic, slide agglutination, and serologic evaluation. Table 3 shows the results of this calculation.

## Discussion

The value of establishing a specific diagnosis of infectious mononucleosis is to distinguish it from other more serious conditions.[15-17] While infectious mononucleosis is often suspected on clinical grounds, the differential diagnosis is not just that of a febrile pharyngitis with lymphadenopathy and malaise. The spectrum of its manifestations includes impaired liver function often with hepatomegaly,[18] hemolytic anemia,[19-21] thrombocytopenia,[22-24] bizarre neurologic complications,[20-23] and immune-complex disease.[25-27] However, in spite of these reports in the literature, complications occur only in one percent of all patients.[13,15,16] In general, infectious mononucleosis is a benign self-limiting disease, so that the efficiency of diagnosis together with the cost effectiveness of diagnostic tests become pertinent.

The purpose of this study was to examine the efficiency and cost effectiveness of making a diagnosis of infectious mononucleosis in two different settings.

In the University Hospital system (Method I), the patients who had an initial positive Monospot test repeated 5 and 11 times merit comment. The patient with five tests did, in fact, have infectious mononucleosis. However, this disease was superimposed on a complex problem involving bloody ascites, hepatosplenomegaly, polycythemia vera, and a hyperfunctional thyroid nodule. Three of the tests were ordered the day following the first Monospot positive test (presumably in error), and the fifth test was ordered eight days later. Confirmatory titers were consistently 1:3,584. On no specific therapy, the infectious mononucleosis resolved over several months.

**Table 3. Cost Effectiveness of Diagnosis***

| Diagnostic Method I | Number of Patients | Cost per positive without confirmatory serologic titering | Cost per positive with confirmatory serology |
|---|---|---|---|
| Patients clinically selected with concurrent WBC, differential, smear, and Monospot† | 1,712 | $272 | $298 |
| | **Incidence of positives** | 6.1% | 5.6% |
| **Diagnostic Method II** | **Number of Patients** | **Cost per positive without confirmatory serologic titering** | **Cost per positive with confirmatory serology** |
| Patients selected by an initial screening based on hematologic criteria in patients suspected of IM. 'Monoscreen' conditional to hematologic criteria.† | 1,969 | $ 58 | $ 63 |
| | **Incidence of positives** | 28.3% | 28.1% |

*Based upon assumed costs of $10.00 for WBC, differential, blood smear
6.50 for Monospot/'monoscreen'
6.50 for differential heterophil titering

† According to Table 1, confirmatory serologic titering was performed only when the Monospot or 'monoscreen' was positive

The patient who had 11 tests repeated had fluctuant heterophil titers over seven weeks; four differential agglutination titers were unmistakably positive at varying times, interspersed by three negative Monospot tests and four weakly reactive titers. The final diagnosis was one of adult juvenile arthritis which became apparent some weeks after the series of Monospot tests. Since cold reactive rheumatoid factors have been detected in the sera of over half of patients with infectious mononucleosis,[28,29] the diagnosis of this patient with a past history of juvenile arthritis was not definitively made until 15 months later when he developed persisting arthritis and subcutaneous wrist nodules with increasing sedimentation rate and ASO titers. An important point in this particular patient was that he had *no* atypical lymphocytes on blood smear even when differential serology was positive for infectious mononucleosis at remarkably high titers.

Repeating the test in the same patient gave a very low yield: only one in 80 patients who had repeated negative Monospot tests resulted in a subsequent positive diagnosis. In this one patient, the first Monospot test (negative) was performed five days after the onset of symptoms, the second (positive), 19 days later. This is consistent with the finding that 40 percent of patients begin to have a rising heterophil antibody five days after the onset of symptoms with the majority of patients reaching maximal titers in the second week.[18] Since the titer bears no relation to the severity of the disease or to the leukocyte changes,[30] repeating a positive test for titer levels is not clinically useful or cost effective unless the clinical situation indicates a repeat testing in relation to timing from the onset of symptoms.

In both groups studied, no adjustment was made for age, since the distribution was in fact comparable. However, it can be said that the stu-

dent population would have a higher incidence of infectious mononucleosis.

A discussion of false positives is not included since the ultimate criterion for infectious mononucleosis is now considered to be the presence of Epstein-Barr antibody.[6]

In both groups studied, differential leukocyte counts and blood smears were performed. However, at the Hall Health Center (Method II), these were performed *before* serologic testing which was conditional to satisfaction of hematology criteria. It seems evident that this prior hematologic screening resulted to a large extent in the fivefold efficiency in diagnosing infectious mononucleosis. As shown in Table 3, this is reflected in a similar improvement in cost effectiveness ($298 reduced to $63). Furthermore, both methods showed that confirmatory titering (differential heterophil/heterophil) resulted in little gain in accuracy of diagnosis and is therefore of limited value.

## Summary

When infectious mononucleosis is suspected clinically, diagnosis is made most efficiently when a leukocyte count is first performed to demonstrate the presence of increased numbers of atypical lymphocytes. This study indicates that by such hematologic selection of patients before serologic testing (whether by Monospot or "monoscreen") a fivefold increase in degree of accuracy of diagnosis can be achieved with corresponding gains in cost effectiveness. A confirmatory heterophil titer adds negligible benefit.

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### References

1. Paul JR, Bunnell WW: The presence of heterophil antibodies in infectious mononucleosis. Am J Med Sci 183:90, 1932
2. Evans AS, Niederman JC, McCollum RW: Seroepidemiologic studies of infectious mononucleosis with EBV. N Engl J Med 279:1121, 1968
3. Banatvala KE, Grylls SG: Serological studies in infectious mononucleosis. Br Med J 3:444, 1969
4. Hampar B, Hsu KC, Martos LM, et al: Serologic evidence that a herpes-type virus is the etiologic agent of heterophil-positive infectious mononucleosis. Proc Natl Acad Sci 68:1407, 1971
5. Evans AS, Niederman JC, Cenabre LC, et al: A prospective evaluation of heterophil and Epstein-Barr virus-specific IgM antibody tests in clinical and subclinical infectious mononucleosis: Specificity and sensitivity of the tests and persistence of antibody. J Infect Dis 132:546, 1975
6. Henle W, Henle G: Epstein-Barr virus and infectious mononucleosis. N Engl J Med 288:263, 1973
7. Ginsburg CM, Henle W, Henle G, et al: Infectious mononucleosis in children: Evaluation of Epstein-Barr virus-specific serological data. JAMA 237:781, 1977
8. Davidsohn I: Serologic diagnosis of infectious mononucleosis. JAMA 108:289, 1937
9. Davidsohn I, Alker PH: The nature of the heterophil antibodies in infectious mononucleosis. Am J Clin Path 5:455, 1935
10. Davidsohn I, Lee CL: The clinical serology of infectious mononucleosis. In Carter RL, Penman HG (eds): Infectious Mononucleosis. Oxford, Blackwell Scientific Publications, 1969, pp 177-200
11. Lee CL, Davidsohn E, Panczyzyn O: Horse agglutinins in infectious mononucleosis: The spot test. Am J Path 49:12, 1968
12. Hoagland RJ: Infectious mononucleosis. Am J Med 13:158, 1952
13. Finch SC: Laboratory findings in infectious mononucleosis. In Carter RL, Penman HG (eds): Infectious Mononucleosis. Oxford, Blackwell Scientific Publications 1969, pp 47-62
14. Wood TA, Frenkel EP: The atypical lymphocytes. Am J Med 42:923, 1967
15. Klemola E, Kaarianen L: Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis. Br Med J 2:185, 1965
16. Klemola E, Von Essen R, Henle G, et al: Infectious mononucleosis-like disease with negative heterophil agglutination test: Clinical features in relation to EB virus and cytomegalovirus antibodies. J Infect Dis 121:608, 1970
17. Fiala M, Heiner DC, Turner JA, et al: Infectious mononucleosis and mononucleosis syndromes: Clinical, virological and immunologic features (medical progress). West J Med 126:445, 1977
18. Finch SC: Clinical symptoms and signs of infectious mononucleosis. In Carter RL, Penman HG (eds): Infectious Mononucleosis. Oxford, Blackwell Scientific Publications, 1969, pp 19-46
19. Deaton JG, Skaggs H Jr, Levin WC: Acute hemolytic anemia complicating infectious mononucleosis: The mechanism of hemolysis. Tex Rep Biol Med 25:309, 1967
20. NeNardo GL, Ray JP: Hereditary spherocytosis and infectious mononucleosis with acquired hemolytic anemia. Am J Clin Path 39:284, 1963
21. Shashaty GG, Atamer MA: Hemolytic uremic syndrome associated with infectious mononucleosis. Am J Dis Child 127:720, 1974
22. Carter RL: Platelet levels in infectious mononucleosis. Blood 25:817, 1965
23. Clarke BF, Davies SH: Severe thrombocytopenia in infectious mononucleosis. Am J Med Sci 248:702, 1964
24. Radel EG, Schorr JB: Thrombocytopenic purpura with infectious mononucleosis. J Pediatr 63:46, 1963
25. Tennant FS Jr: The glomerulonephritis of infectious mononucleosis. Tex Rep Biol Med 26:603, 1968
26. Woodnoffe AJ, Row PG, Meadows R, et al: Nephritis in infectious mononucleosis. Q J Med 43:451, 1974
27. Eaton OM, Little PF, Silver HM: Infectious mononucleosis with pleural effusion. Arch Intern Med 115:87, 1965
28. Capra JD, Winchester RJ, Kunkel HG: Cold reactive rheumatoid factors in infectious mononucleosis and other diseases. Arthritis Rheum 12:67, 1969
29. Goldberg LS, Barnett EV: Mixed yG-yM cold agglutinin. J Immunol 99:803, 1967
30. Wintrobe MM, Lee GR, Boggs DR, et al: Clinical Hematology, ed 7. Philadelphia, Lea and Febiger, 1975, pp 1356-1376