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Original Research Article

Ordering Multiple Serological Tests in the Investigation of Febrile Illness- An Over View

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ABSTRACT

Introduction: Serological reactions are nothing but antigen antibody reactions in vitro. The central dogma of serology is the concept of rise in titre. Laboratory studies must be carefully considered and directed toward establishing an etiologic diagnosis in the shortest possible time, at the lowest possible cost, and with the least possible discomfort to the patient.
Aim: To evaluate the positivity rate of multiple serological tests done for one serum sample.
Materials and Methods: 181 Serum samples that were received for three or more serological tests were processed by rapid test kit methods. The tests were done, to detect 'O' antibodies of Salmonella typhi, and 'H' antibodies of S. typhi, para A & B, anti HCV antibodies, antibodies against chikungunya virus, IgM & G antibodies and NS1 antigen of Dengue virus, HbSAg, anti- malarial antibodies against Plasmodium falciparum & vivax, RA factor, RPR antibodies (antibodies against cardiolipin antigen), CRP and ASO antibodies, in different combinations.
Results: Of the total of 181 samples, majority of them (65.19%) were from patients of less than 30years age group. 85.63% samples were sent for doing three serological tests. About half of the samples (86/181:47.51%) were received from Medicine department, followed by Pediatrics department (19.88%). From the total 181 samples, 584 tests were done. Tests for diagnosis of typhoid fever, dengue fever and hepatitis B infection constituted major portion of serological tests (67.12%). Tests to detect typhoid fever were ordered mostly (137/584; 23.45%), followed by tests to detect hepatitis B infection (129/584; 22.08%) and dengue fever (126/584; 21.57%). Total positivity rate for these 584 serological tests was 10.95%. The percentage of positivity for widal was high (21.89%) followed by CRP (21.15%), ASO (8%), and RA (6.89%).
Conclusions:

1. From the total 181 samples, 584 tests were done.
2. Commonest combination of tests ordered was a combination of Widal, Dengue, HbSAg (31.49%) followed by a combination of tests for Widal, Dengue, Malaria (11.04%).
3. ASO, CRP and RA combination was ordered mostly from orthopedics department and CRP was ordered from pediatrics department.
4. Total positivity rate was 10.95%.
5. The reason for ordering multiple tests was not to miss diagnosis at any cost.

Key words: Infectious diseases, Clinical diagnosis, Laboratory diagnosis, Immunochromatographic assays.

INTRODUCTION

The critical role of the microbiology laboratory in infectious disease diagnosis calls for a close, positive working relationship between the physician and the microbiologists who provide enormous value to the health care team. [1] Laboratory studies must be carefully considered and directed toward establishing an etiologic diagnosis in the shortest possible time, at the lowest possible cost, and with the least possible discomfort to the patient. [2] In a broader context, diagnostic tests can have multiple uses, including: patient management, especially when clinical symptoms are not specific for a particular infection; screening for asymptomatic infections; surveillance; epidemiological; evaluating the effectiveness of interventions; and detecting infections with markers of drug resistance. [3]

Serological reactions are nothing but antigen antibody reactions in vitro. [4] Serological diagnosis of infections with bacterial, viral, fungal or parasitic agents in the clinical laboratory is accomplished by detection of specific antibodies in patient serum specimens. [5] Reactions of antigens and antibodies are highly specific. Because of this high specificity, reactions between antigen and antibody can be used to identify one by means of other. [6] Immunocompetent humans produce both IgM and IgG antibodies in response to most pathogens. [7] IgM is the first antibody to appear after infection. So a serological diagnosis of recent infection may be obtained by performing an IgM specific test on a single serum specimen collected early in the clinical course. [5]

The central dogma of serology is the concept of rise in titre. In the majority of serological procedures for the diagnosis of recent infection, testing both acute and convalescent sera is the method of choice. [7] But it would be difficult to collect convalescent sera, as patients are more interested in immediate diagnosis and treatment rather than waiting for two more weeks for diagnosis. So a single serum sample was collected from patients for serological diagnosis of infectious diseases.

In the laboratory we received single serum samples from febrile cases, for multiple tests in the investigation. That single serum sample was investigated for different serological tests as ordered by the clinicians. We made an attempt to know whether a battery of tests helps clinicians in the laboratory diagnosis of febrile illness or not, and what is the percentage of positivity for these tests and whether clinicians get extra information by doing these tests or not. To the best of our knowledge this study was the first of its kind in our area.

Aim

To evaluate the positivity rate of multiple serological tests done for one serum sample.

Inclusion criteria

Serum samples received for three or more than three serological tests were included.

Exclusion criteria

Haemolysed Serum samples and Serum samples for one or two serological tests were excluded from the study.

MATERIALS AND METHODS

Serum samples that were received at serology section of Microbiology laboratory for more than two serological tests were processed according to standard operative procedures. A total of 181 serum samples, requesting for three and more different serological tests for one sample were received from July to August, 2015. Samples were processed immediately by rapid test kits after receiving them.

Sera for ASO, CRP and RA factor were tested by Latex agglutination test by slide method using kits manufactured by Asritha Diatech India Pvt. Ltd, Hyderabad, India. Test results were read within two minutes. Sera for detection of Salmonella “O” and “H” antibodies were tested by slide agglutination test using kits manufactured by Beacon Diagnostics Pvt. Ltd. Navsari, India. Test results were read within one minute. Malarial antibodies (against

P.falciparum & vivax) were detected by using On site Rapid test (Malaria pf/pv Ab Combo Rapid Test) which is a 3-line, lateral flow immunochromatographic assay, obtained from CTK Biotech simplifying diagnostics, China.

HbSAg was detected by chromatographic immunoassay, using Aspen HbSAg rapid test strip. Anti -HCV antibodies were detected by double antigen sandwich immunoassay, using kits from IND Diagnostic Inc, Canada. Dengue Day 1 test kit for detection of NS1 antigen and IgM & IgG antibodies, manufactured by J. Mitra & Co Pvt. Ltd., New Delhi, India, was used in the laboratory diagnosis of dengue fever. It is a solid phage chromatographic immunoassay. Antibodies against Chikungunya virus were detected by using Chikungunya IgM Combo Rapid test, which is a lateral flow immuno chromatographic assay, from CTK Biotech, USA. Manufacturer's instructions were followed strictly while performing and reading results of the above tests.

RESULTS

A total of 181 samples were received for doing three or more serological tests. Majority of the samples (65.19%) were from patients of less than 30years age group. 85.63% samples were sent for doing three serological tests. About half of the samples (86/181:47.51%) were received from Medicine department, followed by Pediatrics department (19.88%) as shown in Table 1.

Table 1: showing Age, Gender & ward vise distribution of samples

Age	Gender		Number of Tests				Total samples	Single test +ve	Two tests +ve	Med	Surg	Paed	Obg	Ortho	skin	others
	M	F	3	4	5	>5										
<10	12	18	29	1	-	-	30	10	1	-	-	29	-	-	-	1
11-20	20	19	37	2	-	-	39	9	1	23	1	7	1	-	-	7
21-30	21	28	39	5	2	3	49	13	-	23	2	-	5	2	1	16
31-40	10	8	14	3	1	-	18	7	3	10	1	-	1	3	1	2
41-50	11	12	18	3	1	1	23	6	1	14	2	-	-	2	-	5
51-60	7	8	13	1	-	1	15	-	-	11	1	-	-	1	-	2
>60	5	2	5	2	-	-	7	1	-	5	-	-	-	1	-	1
Total	**86**	**95**	**155**	**17**	**4**	**5**		**46**	**6**	**86**	**7**	**36**	**7**	**9**	**2**	**34**
Grand total	**181**		**181**				**181**	**52**		**181**						

Table 2: showing Age & Test vise positivity of samples

Age	Widal		Dengue		HbSAg		Malaria		HCV		Chiku-ngunya		RPR		RA		CRP		ASO	
	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P
<10	29	4	26	5	4	-	10	-	-	-	1	-	-	-	-	-	24	6	1	-
11-20	32	7	33	3	29	1	10	-	3	-	-	-	5	-	1	-	5	-	-	-
21-30	36	9	27	4	40	1	8	-	6	-	2	-	16	-	8	-	7	-	8	-
31-40	10	6	9	1	17	-	2	-	3	-	-	-	5	-	7	2	5	3	4	1
41-50	16	4	13	4	20	-	4	-	1	-	1	-	3	-	7	-	5	1	6	1
51-60	10	-	12	-	12	-	1	-	-	-	-	-	2	-	4	-	4	-	4	-
>60	4	-	6	-	7	-	2	-	-	-	1	-	-	-	2	-	2	1	2	-
Total	**137**	**30**	**126**	**17**	**129**	**2**	**37**	**-**	**13**	**-**	**5**	**-**	**31**	**-**	**29**	**2**	**52**	**11**	**25**	**2**

T= tests; P= Positive

From the total 181 samples, 584 tests were done. Tests for diagnosis of typhoid fever, dengue fever and hepatitis B infection constituted major portion of serological tests (67.12%). Tests to detect typhoid fever were ordered mostly (137/584; 23.45%), followed by tests to detect hepatitis B infection (129/584; 22.08%) and dengue fever (126/584; 21.57%) as shown in table 2.

Table 3: showing Percentage of positivity of different tests

Test	Total tests	No. of Positives	Percentage
Widal	137	30	21.89
Dengue	126	17	5.55
HbSAg	129	2	1.55
RA	29	2	6.89
CRP	52	11	21.15
ASO	25	2	8

Total positivity rate for these 584 serological tests was 10.95%. The percentage of positivity for widal was high (21.89%) followed by CRP (21.15%), ASO (8%), and RA (6.89%) as shown in Table 3.

Commonest combination of tests ordered was a combination of Widal, Dengue, HbSAg (31.49%) and mostly from Medicine department, as typhoid is endemic in this area, Dengue was prevalent during that period and to exclude hepatitis B infection as most of the patients complaining of pain in the right upper abdomen. Next commonest combination of tests was Widal, Dengue, Malaria (11.04%), as typhoid and malaria are endemic in this area and Dengue was prevalent during that period. ASO, CRP and RA combination was ordered mostly from orthopedics department and CRP was ordered from pediatrics department as shown in table 4.

Table 4: showing Department vise distribution of samples in different combinations

Ward	Widal Dengue Malaria (%)	Widal Dengue HbSAg (%)	Widal Dengue HbSAg Malaria (%)	ASO CRP RA (%)	RPR Dengue HbSAg (%)	Widal HbSAg Malaria (%)	CRP Widal Dengue (%)	RPR Widal Dengue HbSAg (%)	RPR HbSAg HCV (%)	RA RPR Widal Dengue HbSAg HCV (%)	ASO CRP RA RPR HbSAg (%)	DOC (%)	Total (%)
Medicine	8	48	6	2	3	3	-	-	2	3	3	9	87(48.06)
surgery	-	-	-	-	2	1	-	-	4	-	-	3	10(5.52)
Paediatrics	8	2	-	1	-	-	10	-	-	2	-	7	30(16.57)
Obg	-	2	-	-	-	-	2	3	-	-	-	-	7 (3.86)
Ortho	-	-	1	10	1	-	-	-	-	-	-	-	12 (6.62)
Skin and STD	-	-	-	-	-	-	-	-	3	-	-	-	3 (1.65)
Other OPs	4	5	2	-	1	-	3	1	-	-	-	16	32(17.67)
Total (%)	**20 (11.04)**	**57 (31.49)**	**9 (4.97)**	**13 (7.18)**	**7 (3.86)**	**4 (2.2)**	**15 (8.28)**	**4 (2.2)**	**9 (4.97)**	**5 (2.76)**	**3 (1.65)**	**35 (19.33)**	**181**

D.O.C.-Different other Combinations

DISCUSSION

The emerging infectious diseases account for 26 per cent of annual deaths worldwide. Communicable diseases account for nearly half of India's disease burden. [8] Early diagnosis of infection is always necessary to provide treatment to patients. [3] Isolation of pathogens is always a definitive diagnosis of infection. But, sometimes lack of culture facilities, risk of occupational hazard, time taken for culture, difficulty in isolation-leads to identification of diseases by serological tests. A serological assay is considered validated if it produces test results that identify the presence or absence of a substance in serum at a specified level of statistical confidence. [9]

In our laboratory we evaluated serum samples that have to be subjected for three and more than three serological tests. These tests were done to detect 'O' and 'H' antibodies of Salmonella typhi, 'H' antibodies of Salmonella paratyphi A & B, anti HCV antibodies, antibodies against chikungunya virus, IgM & G antibodies and NS1 antigen of Dengue virus, HbSAg, anti-malarial antibodies against Plasmodium falciparum & vivax, RA factor, RPR antibodies (antibodies against cardiolipin antigen), CRP and ASO antibodies, in different combinations. As our area is endemic to typhoid fever, malaria and dengue was prevalent during that period, tests to detect these infections were ordered the most. The percentage of positivity of these total tests was 10.95% (584/64). We could not compare our results with other studies as our samples did not represent the actual, total samples for that particular test and more over to the best of our knowledge this study was the first of its kind and we could not found any other similar studies.

The real impact of typhoid fever is difficult to estimate as the clinical picture is confused with those of many other febrile infections. The definitive diagnosis of typhoid fever depends on the isolation of S. typhi from samples. [10] But it takes 2 to 3 days, results in delayed diagnosis and treatment. For this reason, in developing countries typhoid rapid antibody tests can facilitate diagnosis and disease management. [11] The rapid diagnostic tests are more sensitive than blood culture. The Widal test has been used very extensively in the serodiagnosis of typhoid fever and, in developing countries, remains the only

practical test available. [12] Ideally, the Widal test should be run on both acute- and convalescent-phase sera. [13] With a fourfold rise of antibody. However, paired sera are often difficult to obtain and specific chemotherapy has to be instituted on the basis of a single Widal test. Even today, the Widal test remains one of the best, easily accessible, cheap and simple methods for the diagnosis of typhoid fever. [12]

Out of total 181 samples, 137 samples were received for Widal test and 30 samples showed antibody titres of >80 for 'O' antigen and >160 for 'H' antigen of S. typhi. All samples were negative for 'H' antibodies of S. para A & B. Karen H Keddy et al performed widal test for the blood culture positive samples within the subsequent 6 months. [11] In the study of Kulkarni et al 73.3% of typhoid fever cases and 6% of non-typhoidal fever cases showed positivity. [12] Abraham G et al opined that a single Widal test in an unvaccinated patient showing H and/or O titres greater than or equal to 1:160 and typhoid-like symptoms was strongly suggestive of typhoid fever. [14] In our laboratory we considered antibody titres of >80 for 'O' antigen and >160 for 'H' antigen are positive for typhoid fever.

Dengue fever was most prevalent during monsoon seasons. The immature stages of Ae. aegypti are found in water-filled habitats, common during rainy seasons, mostly in artificial containers. Efficient and accurate diagnosis of dengue is of primary importance for clinical care, surveillance activities and outbreak control. [15] Now rapid immunochromatographic tests are available, have the advantage of simplicity and do not require sophisticated equipment. [16] Samples received for detection of NS1 antigen, Ig M & G antibodies of dengue were 126 and 17 samples were positive for Dengue (5.55%). More than 50% of positive cases detected NS1 antigen (9/17). Higher percentage of positivity was seen in Pramiladevi et al study. [17] The authors in another study opined that the clinicians ordered tests to detect NS1 antigen/IgM & G for each and every febrile case, because of fear of missing detection of dengue infection as complications of dengue fever especially in secondary infection are severe. This led to sending of every sample of acute febrile illness for laboratory diagnosis of dengue infection without screening the cases clinically for dengue. [18] The same was true in this study also.

Hepatitis B virus (HBV) infection is a major cause of morbidity and mortality worldwide. [19] HbSAg, if negative, chronic HBV infection is typically ruled out. [20] From total samples, 129 were tested for HbSAg. But only two cases were positive for HbSAg (1.55%). It clearly showed that tests to detect HbSAg were ordered to rule out HBV infection.

C-reactive protein (CRP) is an acute-phase reactant that is synthesized by the liver within six hours after the onset of inflammation and tissue necrosis. [21] The acute-phase response comprises the nonspecific physiological and biochemical responses of endothermic animals to most forms of tissue damage, infection, inflammation, and malignant neoplasia. [22] A CRP value exceeding 20 mg/l has been suggested as a screening limit for bacterial infections. CRP decreases during successful treatment with a half life of three days. The decrease is much more rapid than that of ESR. The rapid decrease of CRP permits the use of this test during the follow up of patients with bacterial infections. [23] Total tests ordered for CRP were 52 and majority from pediatric department (29/52; 55.76%). The positivity in the present study was 21.15% (11/52) and from pediatrics department it was 20.68% (6/29). In present study we were concerned about one time samples, hence we could not comment on follow up. But Mona Nabulsi et al were in the opinion of that the impact of the CRP test results on decision-making is rather small, and CRP ordering may contribute to unnecessary health care expenditures. The current evidence base for CRP testing in pediatric infections is weak and suggests

that CRP is of low diagnostic value. [21]

Clinical diagnosis is imprecise but remains the basis of therapeutic care for the majority of febrile patients in malaria endemic areas. Accurate diagnosis is the only way of effecting rational therapy. [24] Examination of a blood smear by microscopy remains the gold standard for malaria diagnosis. [25] However, microscopy requires well-trained, competent microscopists. [24] Now the available new immunochromatographic antigen capture tests are rapid, easily available, simple to perform and easy to interpret the results. [26] Of the total 181 samples, 37 samples were received for detection of malarial antibodies. But none was positive for malarial antibodies.

Group A streptococci produce many infections, but the two most common are pharyngitis and impetigo. During infection, the host may produce antibodies to one or more extracellular products of group a streptococci, and these antibodies are useful markers of recent streptococcal infection. [27] 25 samples were received for ASO and only two were positive (8%).

Rheumatoid factor was positive in only 70% of patients with RA and present in various other inflammatory diseases and sometimes in healthy persons. [28] The low positive predictive value of the RF casts doubt on the utility of the RF in the diagnostic evaluation of patients. The diagnostic utility of the RF may be greatest when it is negative. [29] Samples received for RA factor were 29 and only two were positive (6.89%).

Usually treatment of patients was largely based on clinical diagnosis. Sometimes clinical examination did not make a specific diagnosis especially in febrile cases where clinical features are same for many infections. In such cases clinicians depends on laboratory diagnosis for specific treatment. Though ordering for more tests is a burden financially for patient if he/she approached a private practitioner or to government in government hospitals, but with no option for clinicians. It was necessary to write for multiple tests, as India is endemic to many bacterial and viral infections, it would be necessary for the clinicians to be open minded for the possibility of any infection. Moreover clinical features of most of infections that cause fever are alike and sometimes clinical features are atypical making clinical diagnosis impossible and made clinicians to seek laboratory diagnosis by ordering for multiple tests. Most of the times they order multiple tests to exclude some infections. Another reason was, for fear of facing litigations from patients if they miss the diagnosis. Sometimes inadequate or incomplete history by the patient, as most of patients is uneducated and they don't know the importance of correct history, also a cause for ordering multiple tests, again not to miss diagnosis.

CONCLUSION

Though ordering multiple tests is a burden economically to patients or to government but it is a must for clinicians, not to miss the diagnosis for the purpose of correct treatment and to exclude some infections, by that patient won't be subjected for unnecessary medication and finally not to land in legal cases for not investigating the case properly.

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