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A New Testing Strategy for the Diagnosis of COVID-19 and Similar Pandemics

COVID-19 Tanısı ve Benzeri Pandemiler İçin Yeni Bir Test Stratejisi

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ABSTRACT Objective: COVID-19 forced the entire world to close all borders yet to work together to contain the pandemic. One dimension of this joint work is sharing medical supplies, promising medications, and diagnostic tools. From the start of the pandemic, one difficulty was to obtain reliable diagnostic tools for this new virus that produces the results in a reasonable time window. We bring a new angle to this strife to increase the performance of a given diagnostic test. **Material and Methods:** In this research, we worked on improving the performance of the RT-PCR (Real Time-Polymerase Chain Reaction) COVID-19 diagnostic test. By obtaining the number of tests conducted and number of positive COVID-19 cases reported by the Ministry of Health of Turkey, using the Bayes' Rule, we predicted the prevalence, the number of false positives and number of false negatives, and we proposed several new testing strategies to improve the COVID-19 test. **Results:** We first presented the single test results. Then we showed that strong negative testing strategies would control the false negative successfully while inflating the false positives. On the other hand, strong positive testing strategy controls the false positives very well while inflating the false negative significantly. Following these results, we have also shown that three-test consensus call strategy perform the best in controlling both false negative and false positive rate. **Conclusion:** To contain COVID-19 or similar epidemics or pandemics, we propose a three-test consensus call strategy, which finds a reasonable balance between false positives and false negatives.

Keywords: COVID-19 diagnosis; sensitivity; specificity; false positive rate; false negative rate

ÖZET Amaç: COVID-19, tüm dünyayı sınırlarını kapatmaya ve pandemiyi kontrol altına almak için birlikte çalışmaya zorladı. Bu birlikte çalışmanın bir boyutu, tıbbi malzemelerin, umut vaadeden ilaçların ve tanı testlerinin paylaşımı olarak kendini gösterdi. Pandeminin daha başından bu yana karşılaşılan zorluklardan biri, bu yeni virüs için güvenilir ve sonuçları makul bir zaman içinde elde edilecek tanı testlerini bulmaktı. Biz bu çabaya, eldeki herhangi bir tanı testinin performansını artıracak yeni bir bakış açısı getiriyoruz. **Gereç ve Yöntemler:** Bu araştırmada, RT-PCR (Gerçek Zamanlı-Polimeraz Zincir Reaksiyonu) COVID-19 tanı testinin performansını artırma üzerinde çalıştık. Türkiye Sağlık Bakanlığı'nın açıkladığı toplam tanı ve pozitif vaka sayılarından hareketle, Bayes Kuralı'nı kullanarak, test edilen kohorttaki COVID-19 prevelansını, yanlış pozitif ve yanlış negatifleri vaka sayılarını hesap ettik, ve COVID-19 tanısını geliştirecek birçok yeni test stratejileri onerdik. **Bulgular:** Önce tekli test sonuçlarını sunduk ve arkasından güçlü negatif test stratejisinin, yanlış pozitifleri artırırken, yanlış negatifleri başarıyla kontrol ettiğini gösterdik. Öbür taraftan, güçlü pozitif test stratejisinin, yanlış pozitifleri çok iyi kontrol ederken, yanlış negatifleri önemli derecede artırmaktadır. Bu sonuçları takiben, üçlü-test konsensus yaklaşımının, hem yanlış pozitifleri hem de yanlış negatifleri kontrol ederek en iyi performansa sahip olduğunu gösterdik. **Sonuç:** COVID-19 ve benzeri epidemi ve pandemileri kontrol altına almak için, yanlış pozitifler ve yanlış negatifler arasında makul bir denge kuran üçlü-test konsensus yaklaşımını öneriyoruz.

Anahtar kelimeler: COVID-19 tanısı; duyarlılık; seçicilik; yanlış pozitif oranı; yanlış negatif oranı

The world is going through historical times since December 31, 2019, when the first cases of New Coronavirus (COVID-19) are officially reported from China. Human Coronavirus (HCoV) is not new to the epidemiology

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world as it was first reported in 1960s[1], Later strains involving serious respiratory tract infections were reported with various names as SARS-CoV in 2003, as HCoV NL63 in 2004, as HKU1 in 2005, and MERS-CoV in 2012.[2] While the international community was aware of this particular virus and its serious potential epidemic potential, its latest version SARSCoV-2 (2019) was portrayed as a typical seasonal influenza and its potential to be a full-blown epidemic than to be a fast spreading pandemic was initially downplayed. The world soon realized its differences as first an epidemic mainly in Wuhan, China and its neighboring regions in South Asia.[3,4] The first case in the United States, which is the most affected country to date, was reported on January 30, 2020[5], and the first case in Turkey was reported on March 10, 2020 (https://covid19.saglik.gov.tr/). COVID-19 was considered to be a pandemic by the World Health Organization (WHO) on March 11, 2020, with a spread history from its starting epicenter Wuhan, China, next to South Korea, Iran, Italy, and through Italy, to the rest of the Europe. As of April 13, the pandemic is strongest in the United States, Italy, Spain, France, Germany, United Kingdom, Iran, and Turkey, where Turkey is 9th in terms of the number of cases and 11th in terms of the COVID-19 deaths.

The main symptoms of the COVID-19 infection were reported to be systemic disorders such as fever, headache, fatigue, sputum production, hemoptysis, acute cardiac injury, hypoxemia, diarrhea, etc., and respiratory disorders including mainly pneumonia, sneezing, cough, sore throat, rhinorrhea, etc.[6] The patients are generally admitted to the hospital with initial diagnosis of pneumonia with unknown etiology.[6]

Among the diagnostic tools of COVID-19 is Chest-computed tomography (CT)[7] and the World Health Organization prepared guidelines for the laboratory testing through Nucleic Acid Amplification Tests (NAAT) such as RT-PCR (Real Time-Polymerase Chain Reaction).[8] National University of Singapore, Saw Swee Hock School of Public Health published a report on COVID-19 diagnostics, in which they described all available and upcoming commercial and non-commercial COVID-19 diagnostic tests.[9] What is surprising is that for most of the tests, no sensitivity and specificity have been provided by the producers of these tests, or unrealistic characteristics such as 100% sensitivity and 100% specificity are reported.[10] Therefore, we will use the only sensitivity measure provided in this report by Fulgent Genetics, USA as 95% and a hypothetical specificity of 90%, which is actually the specificity reported for the IgG and IgM antibody immunoassays.

In this paper, we focus on the laboratory diagnosis of COVID-19 through NAAT and describe the diagnostic characteristics of RT-PCR tests in terms of false positives and false negatives and compare multiple diagnostic strategies to propose more robust laboratory testing approaches in containing the COVID-19 pandemic or other similar epidemics and pandemics we may experience in the future.

MATERIAL AND METHODS

We obtained the number of COVID-19 test conducted and the number of cases reported to be positive from the daily reports of the Ministry of Health of Turkey (Table 1). We computed the positive test ratio with the assumption that the test results are reported in an average of two days; for example, in computing the positive test ratio for March 24, we used the positive cases on March 24 but the number of tests conducted by March 22.

We compare the following testing strategies using sensitivity=0.95 and specificity=0.90. For each new testing strategy, we computed the corresponding sensitivity and specificity. An example of such a computation is given in Appendix Table 1A.

- Single test
- Two tests:
  - Strong Positive: A case is defined to be positive only if both tests are positive. The new sensitivity and specificity are calculated to be 0.9025 and 0.99, respectively.
  - Strong Negative: A case is defined to be negative only if both tests are negative. The new sensitivity and specificity are calculated to be 0.9975 and 0.81, respectively.
- Three Tests:
  - Strong Positive: A case is defined to be positive only if all three tests are positive. The new sensitivity and

TABLE 1: Number of tests and reported COVID-19 positive tests in Turkey.

Date	No. of Tests	No. of Positive Cases Reported	Cumulative Cases	Cumulative Positive Cases	Pos. Percent
On or Before 3/12/2020	20.342	1.236	20.342	1.236	
3/23/2020	3.672	293	24.014	1.529	
3/24/2020	3.952	343	27.966	1.872	9.2%
3/25/2020	5.035	561	33.001	2.433	10.1%
3/26/2020	7.289	1.196	40.290	3.629	13.0%
3/27/2020	7.533	2.069	47.823	5.698	17.3%
3/28/2020	7.641	1.704	55.464	7.402	18.4%
3/29/2020	9.982	1.815	65.446	9.217	19.3%
3/30/2020	11.535	1.610	76.981	10.827	19.5%
3/31/2020	15.422	2.704	92.403	13.531	20.7%
4/1/2020	14.396	2.148	106.799	15.679	20.4%
4/2/2020	18.757	2.456	125.556	18.135	19.6%
4/3/2020	16.160	2.786	141.716	20.921	19.6%
4/4/2020	19.664	3013	161.380	23.934	19.1%
4/5/2020	20.065	3.135	181.445	27.069	19.1%
4/6/2020	21.400	3.148	202.845	30.217	18.7%
4/7/2020	20.023	3.892	222.868	34.109	18.8%
4/8/2020	24.900	4.117	247.768	38.226	18.8%
4/9/2020	28.578	4.056	276.346	42.282	19.0%
4/10/2020	30.864	4.747	307.210	47029	19.0%
4/11/2020	33.170	5.138	340.380	52.167	18.9%

TABLE 1A: Calculations for sensitivity and specificity for three-test consensus call.

D+	Test-1	Test-2	Test-3	Pr-1	Pr-2	Pr-3	No. of + Test	Final Prob.	Decision	P(T+|D+)
P(T+|D+)= 0.95	+	+	+	0.95	0.95	0.95	3	0.857	Positive	0.993
	+	+	-	0.95	0.95	0.05	2	0.045	Positive	
	+	-	+	0.95	0.05	0.95	2	0.045	Positive	
	+	-	-	0.95	0.05	0.05	1	0.002	Negative	
	-	+	+	0.05	0.95	0.95	2	0.045	Positive	
	-	+	-	0.05	0.95	0.05	1	0.002	Negative	
	-	-	+	0.05	0.05	0.95	1	0.002	Negative	
	-	-	-	0.05	0.05	0.05	0	0.000	Negative	
D-	**Test-1**	**Test-2**	**Test-3**	**Pr-1**	**Pr-2**	**Pr-3**	**No. of - Test**	**Step-1**	**Decision**	**P(T+|D+)**
P(T+|D+)= 0.9	-	-	-	0.9	0.9	0.9	3	0.729	Positive	0.972
	-	-	+	0.9	0.9	0.1	2	0.081	Positive	
	-	+	-	0.9	0.1	0.9	2	0.081	Positive	
	-	+	+	0.9	0.1	0.1	1	0.009	Negative	
	+	-	-	0.1	0.9	0.9	2	0.081	Positive	
	+	-	+	0.1	0.9	0.1	1	0.009	Negative	
	+	+	-	0.1	0.1	0.9	1	0.009	Negative	
	+	+	+	0.1	0.1	0.1	0	0.001	Negative	

specificity are calculated to be 0.8574 and 0.999, respectively.

- Consensus Call: Two or more positive tests lead to a 'positive' call and vice versa. The new sensitivity and specificity are calculated to be 0.9928 and 0.9720, respectively.
- Strong Negative: A case is defined to be positive only if all three tests are negative. The new sensitivity and specificity are calculated to be 0.9999 and 0.729, respectively.

For each of these testing strategies, we used an estimated COVID-19 prevalence within the tested cohort as of April 11, 2020, under the single test strategy, which is what is in works in practice, using a search grid that gives the closest number of positive cases to the one reported in Table 1.

All computations in this research were conducted on Microsoft Excel and SAS® Version 9.4.

As we are utilizing publicly available COVID-19 summary data for Turkey, which does not include any human subject data, no Institutional Review Board review is needed for our research. We have conducted this research according to the principles of Helsinki Declaration.

RESULTS

We present the testing characteristics summaries for the total tests conducted for Turkey and a hypothetical new 10,000 tests to compare the numbers of false positives and false negatives more easily across testing strategies.

A disease prevalence of 10.45% gives us a total number of 52,181 positive tests, which is very close to what is reported as of April 11, 2020 (Table 2). Therefore, we will use this as our underlying disease prevalence for the COVID-19 tested cohort. It should be noted that this estimated prevalence is not for the entire population as those tested are not randomly selected individuals but individuals who come to the health-care facilities with some symptoms resembling the symptoms of COVID-19 for which they seek treatment.

With a single-test strategy, for every 10,000 tests, we expect 896 false positives and 52 false negatives. These false negatives may potentially be sent home under the perception that they don't have the virus and can still infect others around them until their symptoms get more severe followed by a positive test or until their infectability period ends, which may be more than two weeks.

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With two-test strategy with strong positive approach (Table 3), for every 10.000 tests, we expect 90 false positives (great reduction from 896 from the single test), and 102 false negatives, which doubled compared to the single-test approach.

With two-test strategy with strong negative approach (Table 4), for every 10.000 tests, we expect 1701 false positives (about twice as many cases compared to the single test), and only 3 false negatives, which seems close to ideal.

With three-test strategy with strong positive approach (Table 5), for every 10.000 tests, we expect only 9 false positives, and 149 false negatives.

With three-test strategy with strong negative approach (Table 6), for every 10,000 tests, we expect 2427 false positives, and no (zero) false negatives.

With three-test strategy with consensus approach (Table 7), for every 10,000 tests, we expect 251 false positives, and only 8 false negatives, which provide a reasonable balance between strong positive and strong negative alternatives.

TABLE 2: Single test strategy.

N Tests	P(T+|D+)	P(T-|D-)	P(D+)	P(T+)	Test +	True +	False +	Test -	True -	False -
276.346	0.950	0.900	0.1045	0.189	52.181	27.434	24.747	224.165	222.721	1.444
10.000	0.950	0.900	0.1045	0.189	1.888	993	896	8112	8.060	52

TABLE 3: Two-test strategy with strong positive approach.

N Tests	P(T+|D+)	P(T-|D-)	P(D+)	P(T+)	Test +	True +	False +	Test -	True -	False -
276.346	0.9025	0.9900	0.1045	0.103	28.537	26.063	2.475	247.809	244.993	2.816
10.000	0.9025	0.9900	0.1045	0.103	1.033	943	90	8.967	8.865	102

TABLE 4: Two-test strategy with strong negative approach.

N Tests	P(T+|D+)	P(T-|D-)	P(D+)	P(T+)	Test +	True +	False +	Test -	True -	False -
276.346	0.9975	0.8100	0.1045	0.274	75.825	28.806	47.019	200.521	200.449	72
10.000	0.9975	0.8100	0.1045	0.274	2.744	1.042	1.701	7.256	7.254	3

TABLE 5: Three-test strategy with strong positive approach.

N Tests	P(T+|D+)	P(T-|D-)	P(D+)	P(T+)	Test +	True +	False +	Test -	True -	False -
276.346	0.8574	0.9990	0.1045	0.09	25.007	24.759	247	251.339	247.220	4.119
10.000	0.8574	0.9990	0.1045	0.09	905	896	9	9.095	8.946	149

TABLE 6: Three-test strategy with strong negative approach.

N Tests	P(T+|D+)	P(T-|D-)	P(D+)	P(T+)	Test +	True +	False +	Test -	True -	False -
276.346	0.9999	0.7290	0.1045	0.347	95.938	28.875	67.064	180.408	180.404	4
10.000	0.9999	0.7290	0.1045	0.347	3.472	1.045	2.427	6.528	6.528	0

TABLE 7: Three-test strategy with consensus call approach.

N Tests	P(T+|D+)	P(T-|D-)	P(D+)	P(T+)	Test +	True +	False +	Test -	True -	False -
276.346	0.9928	0.9720	0.1045	0.129	35.598	28.669	6.929	240.748	240.539	209
10.000	0.9928	0.9720	0.1045	0.129	1.288	1.037	251	8.712	8.704	8

DISCUSSION

In this research, we computed the sensitivity and specificity for several independent replicates of a given diagnostic test with its given sensitivity and specificity and then compared the false positives and false negatives of these new testing strategies. The primary issue with the false negative cases is that these individuals are told that they don't have COVID-19, although they do have it, and this may possibly lead them to be more relaxed in their social interactions and spread the virus to others. The first of the two primary issues with the false positives is that there is an undue psychological burden on these individuals and their families, considering that the death rate due do COVID-19 is at least 6%, which increases by age and other existing comorbidities. The second issue with false positives is that these cases are considered as having the disease and they will need to be quarantined, which may put a huge strain on the healthcare infrastructure which is already overwhelmed by the overall pandemic. Because of these reasons, a practical balance must be found between false positives and false negatives.

We have shown that with the pair of sensitivity and specificity we tested, we incur about 9.5% false decision, mostly false positives. Turkey strived to bring the number of tests conducted in a given day to be beyond 30,000, which is a great achievement; however, it also results in about 2700 false positives and 156 false negatives, if the RT-CPR tests being used had the sensitivity of 95% and the specificity of 90%. While these 2700 false positive cases increase the burden on the healthcare system, 156 false negative cases are not quarantined and thus potentially continue infecting others.

With Two-test Strong Negative Call, while we reduce the false negative to 3 cases in 10,000, it naturally increases the false positives to around 1,700, which means 5,100 false positives in 30,000 tests in a day; such an approach would also create an unnecessary worry in the society and a much greater burden on the healthcare system as these cases must be quarantined. With Two-test Strong Positive Call, on the other hand, we reduce the total mistakes to 192 while the false positives increase from 52 to 102, which is 306 patients in every 10,000 tests. Under this testing strategy, rather than having 52,167 by April 11, 2020, we would have had 28,537 positive cases.

With three-test strong positive, or three-test strong negative calls, we observe again the two extremes of increased false positives or increased false negative. We see that the three-test consensus call find a reasonably balance between the two, with 251 false positives and only 8 false negatives. Therefore, this would be our proposed testing approach.

Once main concern regarding the repeat test in the field is that the testing laboratories are already overburdened by the overflow of the tests every day and repeat testing will only make it worse; however, to be able to harness COVID-19, only such an approach will ease the pandemic speed and the burden on the healthcare system. If we follow such a testing strategy, rather than having 52,167 by April 11, 2020, we would have had 35,598 (68%) positive cases, and only 209 negative cases rather than 1444. Such a reduction in false positive would relieve the quarantine burden on the healthcare system, phycological burden on the patients' families and the COVID-19 worries on the society, and such a reduction on the false negative cases would reduce the spread of the virus significantly.

In practice, three samples are obtained from each suspected patient; two of the are used for RT-PCR and the results are obtained. If both are positive, then it is a positive case; if both are negative, it is a negative case; if one positive and one negative, then the third sample become the tie-breaker for the consensus call. Such a strategy will reduce the number of samples. If the patient is a COVID-19 positive patient, then the need for a third sample processing is about 10%, and if the patient is a COVID-19 negative patient, then the need for a third sample processing is about 18% (Table 1A).

CONCLUSION

We conclude that based on our sensitivity and specificity calculations, a three-test consensus call approach would be a more promising disease-pandemic testing strategy and it is recommended especially for the countries where the COVID-19 cases are still small.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

This study is entirely author's own work and no other author contribution.

REFERENCES

1. Tyrrell DA, Bynoe ML. Cultivation of viruses from a high proportion of patients with colds. Lancet. 1966;1(7428):76-7. PMID: 4158999. [Crossref]
2. Lim YX, Ng YL, Tam JP, Liu DX. Human coronaviruses: a review of virus-host interactions. Diseases. 2016;4(3):26. PMID: 28933406. [Crossref] [PubMed] [PMC]
3. Liu W, Wang F, Li G, Wei Y, Li X, He L, et al. Analysis of 2019 novel coronavirus infection and clinical characteristics of outpatients: an epidemiological study from the fever clinic in Wuhan, China. Lancet. 2/14/2020. Available at SSRN: https://ssrn.com/abstract=3539646 [Crossref]
4. Du Toit A. Outbreak of a novel coronavirus. Nat Rev Microbiol. 2020;18(3):123. PMID: 31988490. [Crossref] [PubMed] [PMC]
5. Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, et al. First case of 2019 novel coronavirus in the United States. N Engl J Med. 2020;382(10):929-36. PMID: 32004427. [Crossref] [PubMed] [PMC]
6. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimm. 2020;109:102433. PMID: 32113704. [Crossref] [PubMed] [PMC]
7. Li Y, Xia L. Coronavirus disease 2019 (COVID-19): role of chest CT in diagnosis and management. AJR Am J Roentgenol. 2020;1-7. PMID: 32130038. [Crossref]
8. World Health Organization (WHO). Coronavirus disease 2019 (covid-19) Situation Report-82; 2020. p.13. https://www.who.int/docs/default-source/corona-viruse/situation-reports/20200411-sitrep-82-covid-19.pdf?sfvrsn=74a5d15_2.
9. He F, Deng Y, Li W. Coronavirus disease 2019: what we know? J Med Virol. 2020 Mar 14. Doi: 10.1002/jmv.25766. Online ahead of print. PMID: 32170865. [Crossref] [PubMed]
10. Yap JC, Ang IY, Tan SH, Jacinta I, Pei CH, Lewis et al. COVID-19 Science Report: Diagnostics. p.30.