Annals of Internal Medicine ORIGINAL RESEARCH

Optimizing SARS-CoV-2 Surveillance in the United States: Insights From the National Football League Occupational Health Program

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Background: Evidence to understand effective strategies for surveillance and early detection of SARS-CoV-2 is limited.

Objective: To describe the results of a rigorous, large-scale COVID-19 testing and monitoring program.

Design: The U.S. National Football League (NFL) and the NFL Players Association (NFLPA) instituted a large-scale COVID-19 monitoring program involving daily testing using 2 reverse transcription polymerase chain reaction (RT-PCR) platforms (Roche cobas and Thermo Fisher QuantStudio), a transcription-mediated amplification platform (Hologic Panther), and an antigen point-of-care (aPOC) test (Quidel Sofia).

Setting: 32 NFL clubs in 24 states during the 2020 NFL season.

Participants: NFL players and staff.

Measurements: SARS-CoV-2 test results were described in the context of medically adjudicated status. Cycle threshold (Ct) values are reported when available.

Results: A total of 632 370 tests administered across 11 668 persons identified 270 (2.4%) COVID-19 cases from 1 August to 14 November 2020. Positive predictive values ranged from 73.0% to 82.0% across the RT-PCR platforms. High Ct values (33 to 37) often indicated early infection. For the first positive result, the median Ct value was 32.77 (interquartile range, 30.02 to 34.72) and 22% of Ct values were above 35. Among adjudicated COVID-19 cases tested with aPOC, 42.3% had a negative result. Positive concordance between aPOC test result and adjudicated case status increased as viral load increased.

Limitations: Platforms varied by laboratory, and test variability may reflect procedural differences.

Conclusion: Routine RT-PCR testing allowed early detection of infection. Cycle threshold values provided a useful guidepost for understanding results, with high values often indicating early infection. Antigen POC testing was unable to reliably rule out COVID-19 early in infection.

Primary Funding Source: The NFL and the NFLPA.

Ann Intern Med. doi:10.7326/M21-0319 **Annals.org**
For author, article, and disclosure information, see end of text.
This article was published at Annals.org on 15 June 2021.

Detection and prevention of transmission of SARS-CoV-2 are critical (1–4), but evidence on effective strategies for surveillance, early detection, and control of SARS-CoV-2 is limited (5, 6). A recent Cochrane review highlighted the uncertainty and low accuracy of various COVID-19 screening strategies in 22 cohort and modeling studies, including temperature measurement, symptom screening, and testing surveillance (7–9). Although many diagnostic tools are available, their utility differs on the basis of baseline test performance, pretest probability, timing of testing, and symptom presentation (10, 11), suggesting that selection of screening strategies and diagnostic tools should be based on context and cost–benefit analysis.

One potential surveillance strategy involves frequent testing for SARS-CoV-2; however, limited data have been published on use of this strategy in a closed population. Published studies of broad surveillance testing strategies have used various diagnostic tests (such as antigen testing, reverse transcription polymerase chain reaction [RT-PCR], and pooling) and frequencies (for example, weekly or twice weekly) and have focused primarily on public health strategies targeting case identification and isolation. Less emphasis has been placed on testing within large surveillance programs to identify true-positive results for isolation and false-positive results to allow return to activity.

Our experience provides unique insight into this real-world testing approach. The National Football League (NFL)/NFL Players Association (NFLPA) COVID-19 Testing and Surveillance Program ("the Program") was designed to enable the start of the 2020 NFL season by implementing comprehensive, evidence-based monitoring protocols (12). Players and staff lived in their home environments while working in NFL facilities and traveling to games. The Program relied on adherence to regular testing and behavioral protocols in addition to contact tracing and other measures to mitigate spread of SARS-CoV-2 (13). Here, we describe the results of this intensive effort and evaluate the utility of daily testing across multiple platforms, including 3 nucleic acid amplification tests (NAATs) and a rapid antigen point-of-care (aPOC) test.

Methods

Monitoring Program

Intake testing was initiated on 13 July 2020, with continuous monitoring in place by 1 August 2020. The NFL regular season, defined by interclub gameplay, started on 10 September 2020. A single laboratory provider, BioReference Laboratories, provided NAAT with 24-hour turnaround for all 32 clubs by using 5 U.S. laboratories. The primary molecular platforms used were Roche cobas (15 clubs), Hologic Panther (11 clubs), and Thermo Fisher QuantStudio (6 clubs), all of which have shown good performance in a clinical setting (14–16). For the purposes of this analysis, NAATs (RT-PCR and

Table 1. National Football League Club Employee Tier Definitions and Associated Testing Cadence

Tier	Definition	RT-PCR Testing Cadence*	aPOC Testing Cadence
Tier 1	Players and essential football personnel (≤60 per club) whose job function requires direct access to players for >10 minutes at a time on a regular basis. Examples include: Coaches Athletic trainers Team physicians Strength and conditioning personnel Equipment managers	1 August to 17 October 2020: Every day except game day 18 October to 14 November 2020: Every day Persons deemed to have an "adjudicated negative" case status (i.e., not found to have COVID-19) continued with daily testing.	aPOC tests administered as needed. Examples included: Case adjudication after initial positive RT-PCR result After invalid prior-day RT-PCR result As part of entry or reentry testing After reported or potential exposure to COVID-19 Newly symptomatic
Tier 2	Other essential personnel (≤40 per club) who may be in proximity to players and other tier 1 persons. Examples include: Club facility staff Ownership/general managers Field managers Video personnel Football operations staff	1 August to 17 October 2020: Every day except game day 18 October to 14 November 2020: Every day Persons deemed to have an "adjudicated negative" case status (i.e., not found to have COVID-19) continued with daily testing.	aPOC tests administered as needed. Examples included: Case adjudication after initial positive RT-PCR result After invalid prior-day RT-PCR result As part of entry or reentry testing After reported or potential exposure to COVID-19 Newly symptomatic
Tier 3	Persons who perform essential facility, stadium, or event services but do not require close contact with tier 1 persons. Examples include: Food/kitchen staff In-house media/broadcast personnel Field maintenance providers Stadium operations staff	Once per week and as indicated, with result available before facility entry (e.g., symptomatic, COVID-19 exposure)	aPOC tests administered as needed. Examples included: Case adjudication after initial positive RT-PCR result After invalid prior-day RT-PCR result As part of entry or reentry testing After reported or potential exposure to COVID-19 Newly symptomatic
Untiered	Staff without access to areas in which players and tiered staff entered. Examples include: Accountants Some IT staff Marketing staff	Upon request	Upon request

aPOC = antigen point-of-care; IT = information technology; RT-PCR = reverse transcription polymerase chain reaction.
* After a positive test result, clubs were instructed to continue to test persons with a positive RT-PCR result until case status could be confirmed. If a case was confirmed, RT-PCR testing ceased. Persons with documented evidence of COVID-19 with RT-PCR confirmation were exempt from testing for 90 days after infection.

transcription-mediated amplification) will be referred to collectively as "RT-PCR" tests. The Quidel Sofia SARS antigen test (17) was used for rapid aPOC testing. Results were categorized as "positive," "negative," "presumptive positive" (Roche cobas), or "inconclusive" (Thermo Fisher QuantStudio). Roche test results (but not Hologic or Thermo Fisher) included numerical values for the target 1 (T1; ORF1) and target 2 (T2; E-gene) cycle thresholds (Cts), which can approximate viral load (18). In addition to routine testing, the Program included ongoing player and staff education, physical distancing and masking requirements, environmental disinfection, and contact tracing protocols to decrease risk for transmission.

Players and staff were placed into 3 predefined tiers (13) based on their anticipated duration of interaction with players (**Table 1**). Players and tier 1 and 2 staff (approximately 80% of the weekly testing population) were tested daily from 1 August through 17 October 2020, except on game day, and transitioned to testing 7 days per week on 18 October due to increasing community prevalence and an incident of within-club transmission (13). Tier 3 staff (about 20% of the weekly testing population) were tested weekly and as indicated (for example, if they were symptomatic or were exposed to COVID-19). Testing ceased once a person's positive result was adjudicated as a COVID-19 case (**Table 1**). Those with documented evidence of prior COVID-19 and RT-PCR confirmation were exempt from testing for 90 days after infection, consistent with Centers for Disease Control and Prevention guidelines (19). Players and staff resided at home in their communities; all participants, including coaches, medical personnel, football operations staff, and facility staff, were required to adhere to testing and behavioral protocols, which enforced precautions such as mask use, distancing, and room capacity restrictions (13). Compliance was monitored daily by the club's infection control officer and NFL League Office staff. Persons who missed a test or were new to the ecosystem were unable to enter the facility until completion of entry testing, which comprised 5 days of RT-PCR testing and a preentry aPOC test on day 6. Symptoms were documented by medical or contact tracing staff.

Results of RT-PCR tests were available within 24 hours of sample collection; aPOC test results were available in less than 1 hour. The NFL/NFLPA testing protocols considered positive, presumptive positive, or inconclusive RT-PCR results as positive cases until further testing and clinical adjudication confirmed or ruled out the case; persons remained in isolation during adjudication (**Table 1** and **Figure 1**). Beginning on 24 August 2020, positive, presumptive positive, and inconclusive samples were

processed a second time on the same platform except at laboratories using the Roche platform, which reprocessed positive samples on the Hologic platform.

In addition to daily RT-PCR testing, aPOC tests were administered for cause in 4 scenarios: to enable clearance for facility entry after an invalid or unavailable RT-PCR result the prior day, after potential exposure to COVID-19, in persons who were newly symptomatic, or for case adjudication (subsequent testing after an initial positive RT-PCR result).

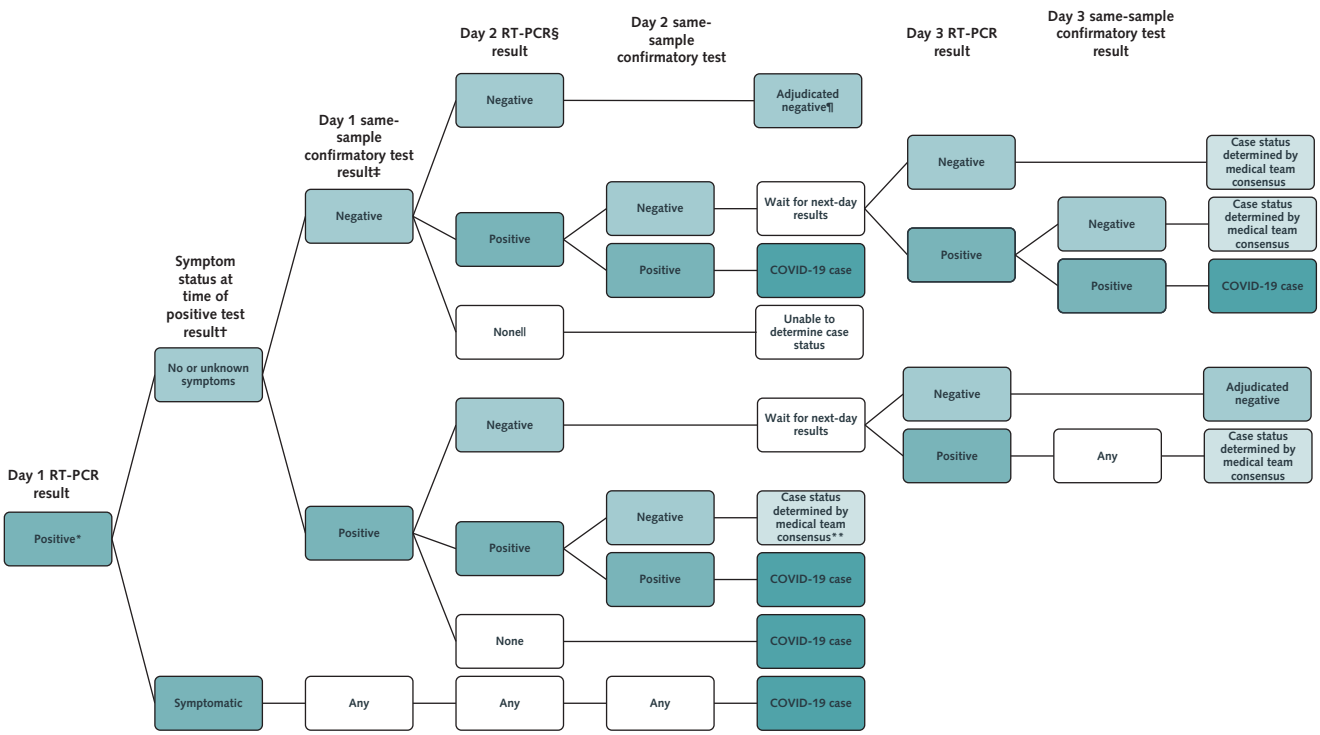
Study Population

The population comprised NFL players and staff employed by a club and undergoing testing as part of the Program between 1 August and 14 November 2020. Players and staff entered and exited the cohort if employment changed; no persons who tested positive were lost to follow-up during the adjudication process due to loss of employment. Sensitivity analyses were performed on 2 additional subgroups: tier 1 and 2 persons (daily testing cadence), and those who reported symptoms at or near receipt of a positive test result.

Outcome Measures

All positive test results were adjudicated by a 4-person panel of epidemiologists and medical experts to determine final case status using multiple, subsequent RT-PCR tests over several days, with new samples collected from the individual, and clinical data. An adjudicated COVID-19 case was defined according to a standardized algorithm (**Figure 1**) by reviewing the initial RT-PCR result; the RT-PCR same-sample confirmatory result (beginning on 24 August 2020); subsequent RT-PCR results; aPOC test results, if available; and symptoms (any positive result

Figure 1. COVID-19 case adjudication algorithm.



RT-PCR = reverse transcription polymerase chain reaction.

* Any "positive" result includes positive, presumptive positive, or inconclusive results.

† If the adjudication team learned of symptoms that presented later, case status was updated to a COVID-19 case, regardless of subsequent test results.

‡ Same-sample confirmatory testing began on 24 August 2020 and was performed for all samples. From 1 August to 23 August, samples with a positive next-day RT-PCR result were considered COVID-19 cases. Positive results required 2 consecutive days of negative follow-up testing to be considered an adjudicated negative case. Any other test result sequence required case determination by medical team consensus. If insufficient follow-up testing was done, the case status was deemed "unable to determine."

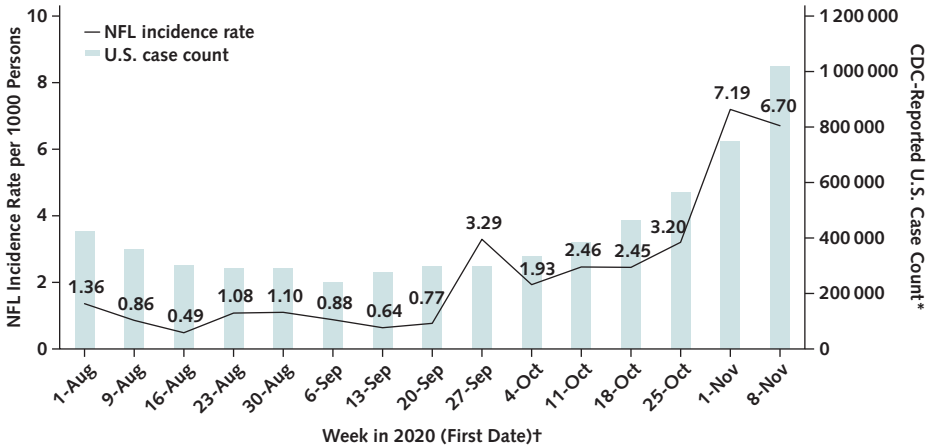
§ Persons with a positive point-of-care test result were largely considered COVID-19 cases, regardless of the same-day confirmatory test result, although these results were discussed by the medical team.

|| If no symptoms were present, clubs were encouraged to do follow-up PCR testing of all asymptomatic/presymptomatic persons, regardless of the confirmatory test result. Sixty-five of 709 initial positive results were among asymptomatic/presymptomatic persons who did not have follow-up testing, 52 of which were adjudicated as COVID-19 cases due to positive same-sample confirmatory test results.

¶ Of 709 initial positive test results, 270 were adjudicated as COVID-19 cases (87 of which were symptomatic at the time of the positive result), 429 were adjudicated as negative, and 10 were deemed "unable to determine."

** Medical team consensus considered testing history, further testing (if available), serologic status, and exposure to other positive persons. All persons reviewed by the medical team were assigned a case status of "COVID-19 case" or "adjudicated negative.".

Figure 2. COVID-19 incidence per 1000 persons, by week, 1 August to 14 November 2020.



CDC = Centers for Disease Control and Prevention; NFL = National Football League.
* Includes cases from all 50 states and the District of Columbia. Data were obtained on 7 May 2021 from https://data.cdc.gov/Case-Surveillance/United-States-COVID-19-Cases-and-Deaths-by-State-o/9mfq-cb36.
† All intervals represent 7-day weeks except the first interval, which represents 1 August to 8 August.

with accompanying symptoms was considered to be confirmation of COVID-19). Persons who were not found to have COVID-19 after a positive result ("adjudicated negative cases") were allowed to return to the facility and continued with daily testing. Given the real-world and real-time nature of testing, tracing, and isolation, viral culture was not available to supplement case adjudication.

Advarra Institutional Review Board approved this analysis, which was conducted in accordance with the NFL/NFLPA Medical Research Approval Protocol (20).

Statistical Analysis

All analyses were descriptive; no hypotheses were tested. Missing data were not imputed. Incidence rates were calculated as the number of new adjudicated cases out of the total number of persons tested in the period of interest. Distribution of Ct values for initial positive test results is presented for results obtained from the Roche cobas instrument. Analyses of Ct values focus on T1 (ORF1); T2 is described if T1 was negative.

RT-PCR Test Results and Adjudicated COVID-19 Case Status

Positive results from RT-PCR platforms collected from 24 August through 14 November 2020 were evaluated against adjudicated case status; the positive predictive value (the proportion of initial positive results that resulted in an adjudicated case of COVID-19) (21) was calculated. Only 1 result per testing series from the adjudication process (the initial positive result from an individual) was analyzed; follow-up RT-PCR tests after an initial positive result, often performed for confirmatory purposes, were excluded. Positive concordance (the proportion of positive results with a same-sample positive result) (22) was evaluated for first and repeated results performed on the same sample.

aPOC Test Results

Results of aPOC tests were compared against same-day RT-PCR results and the final adjudicated case status (1 aPOC test per case). Concordance between the same-day aPOC test result and the RT-PCR result was described. Positive concordance was defined as the proportion of positive RT-PCR results with a positive aPOC test result, and negative concordance was defined as the proportion of negative RT-PCR results with a negative aPOC test result (22). Among persons with adjudicated COVID-19 cases, we report the proportion with negative and positive aPOC test results, as well as Ct values for same-day RT-PCR (Roche only), and the proportion of positive aPOC test results among noncases.

Role of the Funding Source

Protocols, program conduct, operations, analytics, and manuscript preparation for this COVID-19 monitoring program were funded by the NFL/NFLPA.

RESULTS

Test Volume, Case Characteristics, and Incidence Rate

Between 1 August and 14 November 2020, a total of 632 370 RT-PCR tests (46.2% on the Roche platform, 34.8% on the Hologic platform, and 19.0% on the Thermo Fisher platform) and 13 804 aPOC tests were administered to 11 668 persons (7000 to 8000 persons per week); 270 (2.4%) cases were adjudicated as COVID-19 cases. Weekly incidence in the NFL fluctuated between 4 and 11 cases in August and September, then increased to 15 to 26 cases in October and 52 to 56 cases in November. The weekly incidence rate per 1000 persons increased from less than 3.5 in August to 7.2 in November (Figure 2), parallel to increasing community incidence during this period (23). Of 709 initial positive RT-PCR

results, 98.6% were adjudicated to a final case status; 10 (1.4%) were not adjudicated due to insufficient follow-up testing, all among staff. Among infected persons for whom symptom status was reported (n = 186 out of 270 adjudicated COVID-19 cases), approximately half (53.2% [n = 99]) reported having no symptoms at or near receipt of a positive test result; this percentage was stable (54.7% [n = 82 of 145]) when restricted to tier 1 and 2 persons (daily testing cadence). Symptoms reported early in infection included headache (n = 21 [27%]), cough (n = 19 [25%]), fever (n = 17 [22%]), nasal congestion (n = 18 [23%]), sore throat (n = 17 [22%]), chills (n = 14 [18%]), body aches (n = 13 [17%]), and loss of taste and/or smell (n = 9 [12%]).

RT-PCR Results

Table 2 presents positive predictive values for initial positive results by platform as well as concordance statistics on same-sample test results from 24 August to 14 November 2020. The positive predictive values for RT-PCR ranged from 73.0% to 82.0%. Positive same-sample concordance for samples processed on the same machine was approximately 75%. Analyses restricted to tier 1 and 2 persons showed similar results; when analyses were restricted to cases with symptoms at the time of a positive test result, same-sample concordance increased to more than 90% (Appendix Table, available at Annals.org).

For presumptive positive and positive samples on the Roche platform that were subsequently tested on the Hologic platform (n = 182), 53.8% (n = 98) returned negative results on the Hologic platform. Among these, 33 (33.7%) were adjudicated as COVID-19 cases after subsequent testing and clinical review, 85% of which (n = 28) had Ct values above 35.0. In weeks of higher incidence (25 October to 14 November), the proportion of samples that were adjudicated as COVID-19 cases after a positive or presumptive positive result on the Roche platform but a negative result on the Hologic platform was 66.7% (24 of 36).

Among the 28 persons who had symptoms at the time of a positive result and were tested on the Roche platform, all were adjudicated as COVID-19 cases per protocol; however, 2 returned negative results on the Hologic platform on the day of the first positive result. Both platforms showed positive results on subsequent days.

Initial Ct Values at the Time of Case Identification

Roche was the only quantitative machine used; Ct T1 values were available for 113 adjudicated COVID-19 cases from 1 August through 14 November. The mean Ct T1 value from the first positive test result for adjudicated COVID-19 cases was 30.45 (SD, 4.81) (median, 31.53 [interquartile range {IQR}, 27.55 to 34.12; range, 17.65 to 37.44]).

When the analysis was restricted to persons with a negative RT-PCR result the day before the initial positive result (n = 72; excludes persons not tested with RT-PCR the day before their positive result for tier or scheduling reasons), Ct values for T1 were slightly higher, with 75% above 30, 22% above 35, and a mean of 32.10 (SD, 3.68) (median, 32.77 [IQR, 30.02 to 34.72; range, 18.34 to 37.44]). Only 3 (4%) of these had a T1 Ct value below 25. For presumptive positive results adjudicated as COVID-19 cases (Ct T1 negative; n = 13), the T2 Ct values ranged from 35.57 to 39.72, with a mean of 37.57 (SD, 1.15) and a median of 37.79 (IQR, 37.35 to 37.90).

We performed a sensitivity analysis on 33 adjudicated COVID-19 cases with symptoms at the time of a positive test result (29.2% of 113 Roche cases total). Similar to the full Roche cohort, the mean T1 Ct value from the first positive result for adjudicated symptomatic COVID-19 cases was 29.82 (SD, 5.28) (median, 32.09 [IQR, 26.15 to 33.74; range, 17.65 to 36.93]).

Serial testing was critical for interpretation of Roche test results with initial high Ct values. Overall, 61 persons had an initial positive result with a T1 Ct value above 35; 19 (31.2%) were ultimately adjudicated as COVID-19 cases. Of these, 14 had RT-PCR testing on the next day with a positive result; 12 (85.7%) had a

Table 2. RT-PCR Results With Adjudicated Status, by Platform, 24 August to 14 November 2020

Variable	Initial Platform				
	Roche (n = 182)		Hologic (n = 95)	Thermo Fisher (n = 69)	
Initial result, n*	Positive: 137	Presumptive positive: 45	Positive: 95	Positive: 61	Inconclusive: 8
Confirmed cases, n†	100	13	73	50	4
Positive predictive value (95% CI), %	73.0 (65.6–80.4)	28.9 (15.6–42.1)	76.8 (68.4–85.3)	82.0 (72.3–91.6)	50.0 (15.4–84.6)
	Same-Sample Confirmatory Test Platform‡				
	Hologic		Hologic	Thermo Fisher	
Positive result on confirmatory test (second run), n (%)§	84 (61.3)	0 (0)	71 (74.7)	48 (78.7)	1 (12.5)

RT-PCR = reverse transcription polymerase chain reaction.

* Excludes persons with a documented prior COVID-19 case (confirmed by PCR testing), those without enough follow-up testing to determine a case status, and 1 person with an initial test on the Hologic platform and a retest on the DiaSorin test platform due to technical issues. Analyses of positive test results were restricted to the first positive result for an individual; follow-up tests during infection were excluded from analyses.

† All cases were adjudicated by a panel of epidemiologists and medical experts (see Figure 1).

‡ On the Thermo Fisher platform, 3 initial positive results and 7 inconclusive results were inconclusive on same-sample processing; 2 and 3 resulted in adjudicated COVID-19 cases, respectively.

§ Analyses were restricted to initial positive test results that had a same-sample confirmatory test. As of 24 August, all positive, presumptive positive, and inconclusive samples were tested a second time as a confirmatory test (i.e., the same specimen was tested again from the primary collection tube) as follows: Samples with a positive result on the Thermo Fisher (6 clubs) and Hologic (11 clubs) platforms were run on the same instrument as the initial test, and samples with a positive result on the Roche platform (15 clubs) were run on the Hologic platform.

Table 3. Sofia SARS aPOC Rapid Test Result Compared With Clinically Adjudicated Case Status and Same-Day RT-PCR Result, 1 August to 14 November 2020

Variable	Adjudicated COVID-19 Case Status		Total
	Positive*	Negative	
aPOC test result, *n*			
Positive	75	39	114
Negative	55	11 882	11 937
Total	130	11 921	12 051
	Same-Day RT-PCR Result		**Total**
	Positive	**Negative**	
aPOC test result, *n*†			
Positive	89	40	129
Negative	85	10 768	10 853
Total	174	10 808	10 982

aPOC = antigen point-of-care; RT-PCR = reverse transcription polymerase chain reaction.

* Evaluated per initial positive PCR result through case adjudication (Figure 1). Only 1 aPOC test result was included per case; aPOC testing was typically done the day after the initial positive result on RT-PCR (88%). If no next-day aPOC sample was available, samples from the same day (9%), the day before (1%), or 2 days after (2%) were used. In cases with multiple aPOC tests with different results on the same day (e.g., 1 negative and 1 positive), only 1 test with a negative result was included. In cases with multiple aPOC tests with the same result on the same day (e.g., 2 positives), only 1 test was counted here.

† Indicates agreement between RT-PCR and aPOC; does not reflect final case status. Because of these exclusions, the number of aPOC tests analyzed is fewer than the 12 051 tests with an adjudicated case status.

subsequent decrease in Ct value below 35 for that next-day test.

aPOC Test Results

Of 13 804 Sofia aPOC tests performed, 12 051 had a valid result and were included in the analysis; 1753 were excluded because of an invalid result, a data transmission error, or multiple tests for 1 COVID-19 case. Among aPOC tests administered, 68% were to persons with a negative RT-PCR result the day before and 28% were to persons with no RT-PCR test the day before; the remainder were administered the day after a positive RT-PCR result (3%) or an invalid RT-PCR test (1%). A same-day RT-PCR sample was collected for 10 982 aPOC tests.

Positive concordance on same-day aPOC and RT-PCR results was 51.2%, and negative concordance was 99.6% (**Table 3**). Among the 130 adjudicated COVID-19 cases with aPOC tests administered, 55 (42.3%) tested negative on aPOC and 75 (57.7%) tested positive. In addition, 34% ($n = 39$) of positive aPOC test results were determined to not be COVID-19 cases. Sensitivity analyses restricted to persons in tiers 1 and 2 (that is, those who were tested daily) had consistent results, with 41.0% of adjudicated COVID-19 cases testing negative on aPOC (**Appendix Table**). Similarly, sensitivity analyses limited to symptomatic persons showed a slightly smaller proportion (34.8% vs. 42.3%) of adjudicated COVID-19 cases testing negative on aPOC (**Appendix Table**).

Forty persons with adjudicated COVID-19 cases had both an aPOC test administered and a Roche RT-PCR sample collected on the same day. In 17 of these cases, the aPOC test result was positive (that is, it detected the infection), with a mean T1 Ct value of 23.62 (SD, 3.86) and a median of 23.88 (IQR, 21.85 to 26.40). In the other 23 adjudicated COVID-19 cases, the aPOC test result was negative, with a mean same-day Roche Ct value of 31.46 (SD, 2.90) and a median of 30.86 (IQR, 29.68 to 33.47).

DISCUSSION

The intensive surveillance and mitigation work performed during the 2020 NFL season provided 4 key findings. First, frequent, routine RT-PCR testing protocols enabled early detection of infection, often when the person had low viral load and no symptoms. Second, when the same sample was analyzed on 2 different platforms, there were instances of discordant results, indicating variability of test performance. Third, Ct values produced by quantitative platforms can be useful in interpreting positive test results within a large, routinely tested population, with high Ct values potentially able to signal early infection. Fourth, in this setting, the Quidel Sofia aPOC test was unable to detect infection in its early stages in many instances, with negative results in 42% of adjudicated COVID-19 cases.

Testing with RT-PCR identified cases early in infection in our study, when viral loads were likely low, and for more than 50% of cases when no symptoms were present. These findings align with a meta-analysis of 16 NAAT performance studies (8) summarizing data from 5922 ambulatory and outpatient samples with 941 (16%) positive results and often with mild or no symptoms. A combined sensitivity of 84.8% (95% CI, 76.8% to 92.4%) and specificity of 97% (CI, 93% to 99%) for nasopharyngeal swab NAAT was observed, indicating good performance. Together, these studies illustrate the utility of RT-PCR testing for broad population surveillance among asymptomatic and presymptomatic persons, allowing early isolation to minimize exposure. In the NFL Program, RT-PCR–based testing strategies combined with mitigation measures and contact tracing prevented secondary transmission of infection (13).

Our study provides unique data on different RT-PCR platforms when used as part of a screening program and reports concordance of same-sample processing on 2 different platforms, although comparisons were available only for samples analyzed when the Roche platform returned an initial positive result and then subsequently processed on the Hologic platform. In this population of infection-naive persons with frequent testing, the Roche platform was able to identify new, early-onset infections. Among these adjudicated COVID-19 cases, the Hologic platform returned a negative result 33.7% of the time, and this increased to 66.7% during increased COVID-19 incidence. Both the Roche and Hologic platforms returned positive results for these persons during follow-up testing later in infection. It is important to note that cases were adjudicated on the basis of serial diagnostic test results alongside clinical assessment consistent with methods recommended by the U.S. Food and Drug Administration and by longitudinal monitoring of all

persons in these cohorts rather than by viral culture (24); thus, false positives cannot be ruled out among adjudicated cases. Given that initial positive results on the Hologic platform were processed on this platform only and not on the Roche platform, we are unable to comment on instances where initial positive results on the Hologic platform may have been negative on the Roche platform.

Clinical interpretation of high Ct values (>35) from positive SARS-CoV-2 results on RT-PCR has been debated. Some have suggested that Ct values above 35 may not signify infectious cases but rather reflect testing during the noninfectious recovery phase (25–28), whereas others have suggested that high Ct values indicate early infection (29, 30). Our data support the latter and suggest that interpreting these as noninfectious could lead to missed diagnoses and opportunities to mitigate transmission. In the NFL Program, as these data came to light and persons with a high Ct value were subsequently adjudicated as COVID-19 cases, safety precautions surrounding initial interpretation of high-Ct positive results shifted to preventive isolation pending additional testing and adjudication despite negative aPOC test results. Additional considerations, such as clinical course, infection history, exposure, and serologic results, can help interpretation of Ct values to distinguish between early infection versus viral shedding (18, 31–34). The NFL experience can inform surveillance in other settings of closed populations with potentially vulnerable persons, such as nursing homes and correctional facilities, where detection and isolation of persons with early infection are particularly critical.

Among persons with COVID-19 who had an aPOC test, we found that the result was negative 42.3% of the time. This is concerning because aPOC tests were often administered for cause (for example, when new symptoms developed, after exposure, or after a positive RT-PCR result). On the basis of these findings, aPOC testing was replaced with PCR-based POC tests for the second half of the NFL season. These findings align with a review of 78 studies and 24 087 samples (7415 cases), which concluded that rapid antigen tests had high specificity but variable sensitivity ranging from 33% to 87%; sensitivity was higher among symptomatic participants and those with lower Ct values (≤25), which also is consistent with our results. The authors of the review estimated that 12.5% to 25% of cases could be missed in high-prevalence (5%) settings and 33.3% to 50% could be missed in low-prevalence (0.5%) settings (8); this is consistent with our finding that 42% of adjudicated COVID-19 cases tested negative on aPOC (among those given an aPOC test). A study among schoolchildren estimated positive RT-PCR concordance of 56%, which increased to 64% when the children were symptomatic and 94% when Ct values were 25 or lower (22). Antigen POC tests in addition to postexposure quarantine, and ideally RT-PCR confirmation, may be important for-cause testing in low-prevalence, presymptomatic populations (3, 35), with overall use of aPOC tests requiring further validation and context of pretest probability and individual circumstances.

This study is among the first to summarize the use of daily RT-PCR testing for COVID-19 surveillance. Other published examples of surveillance programs have focused on less frequent and/or pooled testing in academic or nursing home settings (8, 9, 36, 37) or in the setting of outbreaks (38, 39). Our results provide unique insights into specific diagnostics and a more intensive testing approach and the importance of an adjudication strategy that quickly identifies both persons with new-onset COVID-19 and potential false-positive results in order to allow prompt return to work. Lessons learned from this return-to-work monitoring program can inform business and population-wide efforts. Ongoing analyses and review of test characteristics by medical experts drove real-time, evidence-based modifications to testing protocols and other mitigation efforts, including interpretation of high-Ct positive results and use of aPOC testing to rule out infection. Real-time evaluation of testing strategies, in addition to these protocol changes, led to the ability to complete an NFL season despite high prevalence in communities. These findings and similar analyses will be key to the community at large in the development of effective surveillance strategies for return to activity.

Our study has limitations. Analyses of RT-PCR tests relied on laboratory-reported data designed for clinical use, and assignment of platforms was not randomized and included procedural differences across the 5 laboratories; nevertheless, this reflects routine real-world clinical testing conditions. Although positive test results were carefully adjudicated and data quality was reviewed, negative results did not undergo the same level of scrutiny. Our study also relied on diagnostic test results and clinical assessment rather than viral culture for determining infection, potentially resulting in some misclassification. Comparisons across all 3 RT-PCR platforms are limited because samples initially tested on Thermo Fisher or Hologic were not reprocessed on different platforms, and comparisons between Roche and Hologic were unidirectional. These results may also not be generalizable to COVID-19 variants, which were less prevalent in this time frame. Finally, the strategies used in the NFL Program were resource-intensive and may not be feasible in all settings. Ongoing evaluation of these diagnostics in intensive monitoring settings is needed, including additional research on the utility of Ct values, the implications of testing persons who have recovered from COVID-19, and the introduction of vaccinations and new variants.

In conclusion, frequent RT-PCR surveillance used in the NFL Program allowed for detection of new infections, including those early in the clinical course. Quantitative values proved useful for understanding test results, with high Ct values signaling early infection. The aPOC test showed a high rate of false results among adjudicated COVID-19 cases and was not able to consistently detect infection, necessitating protocol and operational changes. These findings inform our understanding and development of population-level SARS-CoV-2 testing and surveillance strategies to mitigate spread during the pandemic.

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Disclaimer: The views expressed in this article are those of the authors.

Acknowledgment: The authors thank the NFL club infection control officers; the NFL team athletic trainers, physicians, and orthopedists; the NFLPA; Kalyani Hawaldar and Kristina Zeidler for their analytic input; and the NFL and IQVIA contact tracing teams.

Financial Support: By the NFL and the NFLPA.

Disclosures: Disclosures can be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M21-0319.

Reproducible Research Statement: *Study protocol:* NFL/NFLPA COVID-19 testing protocols are available at www.nfl.com/playerhealthandsafety/health-and-wellness/covid-19/nfl-nflpa-covid-19-protocols-for-the-2020-regular-season. *Statistical code:* Not available. *Data set:* Not available as governed by collective bargaining agreement. The NFL/NFLPA medical research approval process is described in reference 20.

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Appendix Table. Sensitivity Analyses: Examination of Tier 1 and 2 Persons and Those Who Reported Symptoms at or Near Receipt of a Positive Test Result

Variable	RT-PCR Results With Adjudicated Status, by Platform, 24 August to 14 November 2020 (Table 2)				
	Roche: Positive	Roche: Presumptive Positive	Hologic: Positive	Thermo Fisher: Positive	Thermo Fisher: Inconclusive
Positive predictive value (95% CI), %					
Full population	73.0 (65.6–80.4)	28.9 (15.6–42.1)	76.8 (68.4–85.3)	82.0 (72.3–91.6)	50.0 (15.4–84.6)
Restricted to tier 1 and 2 persons*	67.6 (58.7–76.6)	26.3 (12.3–40.3)	75.0 (65.5–84.5)	83.6 (73.9–93.4)	50.0 (15.4–84.6)
Positive result on same-sample confirmatory test (second run), %					
Full population	61.3	0	74.7	78.7	12.5
Restricted to tier 1 and 2 persons*	53.3	0	72.5	80.0	12.5
Restricted to symptomatic persons†	92.9	0	92.6	95.0	0

	Sofia SARS aPOC Rapid Test Result Compared With Clinically Adjudicated Case Status and Same-Day RT-PCR Result (Table 3)				
	Positive Concordance With Same-Day RT-PCR	Negative Concordance With Same-Day RT-PCR	Adjudicated Cases With Positive aPOC Test Result	Adjudicated Cases With Negative aPOC Test Result	Positive aPOC Test Results Adjudicated as Negative Cases
Full population, %	51.2	99.6	57.7	42.3	34.2
Restricted to tier 1 and 2 persons, %*	53.6	99.7	59.0	41.0	28.9
Restricted to symptomatic persons, %†	65.1	NA	65.2	34.8	NA

aPOC = antigen point-of-care; NA = not applicable; RT-PCR = reverse transcription polymerase chain reaction.
* Excludes tryout players in addition to tier 3 and untiered staff.
† Symptomatic at or near time of positive test result.