

Traditional versus reverse syphilis algorithms: A comparison at a large academic medical center



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ABSTRACT

Objectives: An increasing number of institutions are transitioning from the traditional syphilis testing algorithm (initial screening with nontreponemal tests) to the ‘reverse’ algorithm (initial screening with treponemal tests such as syphilis IgG). The aim of this study was to evaluate the switch in syphilis algorithm at an academic medical center with a population with low syphilis prevalence.

Design and methods: We performed a six-year retrospective study at the University of Iowa Hospitals and Clinics, an academic medical center, comparing the traditional algorithm (n = 12,612) with the reverse algorithm (n = 10,453). False positives were considered to be positive screens with negative confirmatory testing.

Results: Using the traditional algorithm, 93 samples (0.7% of total) screened positive with RPR, with 40 of these samples having negative TP-PA testing (43% of positive screens, 0.3% of total). Using the reverse algorithm, 110 screened positive with syphilis IgG (1.1% of total), and 33 of these samples had both negative RPR and TP-PA (30% of positive screens, 0.3% of total). In both algorithms, higher RPR titers and syphilis IgG values were associated with increased probability of positive confirmation.

Conclusions: In this study at an academic medical center, the reverse algorithm had significantly more total positive screens than the traditional algorithm. Both algorithms produced equivalent rates of active infection. The quantitative difference in positives between the two algorithms are the category of patients who are syphilis IgG positive, RPR non-reactive, and TP-PA reactive. Specimens with higher RPR titers and syphilis IgG values are more likely to confirm positive.

1. Introduction

Serologic testing has been the standard for syphilis diagnosis. Serologic testing is divided into two major groups: treponemal, which tests for specific antibodies to *Treponema pallidum* (the causative organism), and nontreponemal, which tests for antibodies formed in response to cellular damage [1]. The use of nontreponemal or treponemal tests in isolation is not ideal as each type of test has limitations; hence, there are two commonly used approaches for the serologic diagnosis of syphilis – traditional and ‘reverse’ algorithms [2].

The traditional testing algorithm for syphilis begins with a screening nontreponemal test such as rapid plasma reagin (RPR), with positive results followed by a confirmatory treponemal test such as fluorescent treponemal antibody or *T. pallidum* particle agglutination assay (TP-PA) [3]. RPR titers are sensitive, decrease with treatment, and have traditionally been more convenient and less expensive to perform than treponemal tests, rationalizing this algorithm. With increasing automation and decreasing cost, an

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increasing number of institutions have adopted a reverse testing algorithm which first screens with a treponemal test (e.g., syphilis IgG); reactive samples are then tested by RPR which is used to assess disease activity [4]. Discordant syphilis IgG and RPR results are resolved by a second treponemal test (e.g., TP-PA), as recommended by the Centers for Disease Control and Prevention (CDC) for laboratories adopting the reverse algorithm [5]. In the setting of a positive syphilis IgG and non-reactive RPR, a nonreactive treponemal test result indicates a false positive syphilis IgG screen because TP-PA has higher analytical sensitivity than syphilis IgG screening [6]. Conversely, a reactive result suggests prior treated syphilis or late/latent syphilis for which the CDC recommends treatment to decrease possibility of developing tertiary syphilis [5].

Per the above discussion, it is important to distinguish analytical false positive results from clinical false positive results. A positive syphilis IgG screen with negative RPR and TP-PA confirmatory testing can be considered an analytical false positive. A positive syphilis IgG with positive TP-PA and negative RPR may be an analytical false positive due to cross-reacting antibodies or an analytical true positive result in late/latent syphilis or past/treated syphilis with persistent anti-syphilis IgG. Patients with or without signs and symptoms of syphilis and a positive/negative/positive set of results are empirically treated given that there is no definitive gold standard for diagnosis of latent syphilis, treatment with penicillin G benzathine is inexpensive, and personal and public health consequences of a missed opportunity to treat can be large. For the reverse algorithm, we therefore refer to syphilis IgG-positive, RPR-negative, TP-PA negative patients as “false positive”; and syphilis IgG-positive, RPR-negative, TP-PA positive patients as “positive”. For the traditional algorithm, we refer to a reactive RPR with a nonreactive TP-PA as “false positive”, and a reactive RPR with positive TP-PA as “positive”.

As defined above, multiple studies have noted an increased rate of false positives with the reverse compared to the traditional syphilis algorithm [4,7–10]. This represents one of the major potential limitations of the reverse algorithm [11]. False positives can cause anxiety for providers and patients and potentially lead to unnecessary treatment. Previously undetected patients with positive syphilis IgG, negative RPR, and positive TP-PA also contribute to the burden of anxiety and treatment. Though there is no gold-standard test to adjudicate these results, this set of patients is more likely to have syphilis if they belong to a population where syphilis is more prevalent.

The University of Iowa Hospitals and Clinics (UIHC), a state academic medical center, switched from the traditional to the reverse syphilis algorithm in February 2013. According to data from the CDC, the incidence of primary and secondary syphilis in the state of Iowa has increased from 0.8 per 100,000 to 3.4 per 100,000 from 2009 to 2013, with the increase primarily in men who have sex with men [12], similar to overall national trends [13]. There were no cases of congenital syphilis reported in Iowa from 2009 to 2013 [12]. In the present study, we performed a six year retrospective analysis of syphilis testing results before and after the switch to the reverse syphilis algorithm at UIHC. A main focus of our study was how the reverse algorithm performed over a multi-year timeframe with respect to the distribution of positive and false positive results. A secondary aim was to evaluate whether higher syphilis IgG quantitative values and RPR titers associated with increased rates of positive TP-PA testing.

2. Materials and methods

2.1. Study population

With approval from the University of Iowa Institutional Review Board (protocol # 201501705), a retrospective study comparing results from the traditional syphilis algorithm (n=12,612 screening tests; May 1, 2009 to February 24, 2013) with the reverse algorithm (n=10,453 screening tests; February 25, 2013 to October 12, 2015) was performed for UIHC (see Fig. 1). UIHC is a 734 bed tertiary care academic medical center that includes an emergency department, adult and pediatric inpatient floors, and multiple intensive care units (cardiovascular, medical, neonatal, pediatric, and surgical/neurologic). Primary care and specialty outpatient clinics are provided at the main medical campus as well as at clinics throughout the local region. UIHC is a regional center for high-risk obstetric and neonatal care.

2.2. Laboratory analyses

Syphilis IgG testing was performed on a Bioplex 2200 analyzer (Bio-Rad Laboratories, Hercules, CA, USA) using the Syphilis IgG (*T. pallidum*) assay in the UIHC core clinical laboratory. Samples are run 24 h a day and results can auto-verify if quality criteria are met [14]. Following the information in the assay package insert, the following were the reference ranges for the syphilis IgG assay using units of antibody index (AI): 0.8 AI or lower, negative; 0.9–1.0 AI, equivocal; 1.1 AI or greater, positive. RPR and TP-PA tests were referred to a commercial reference laboratory (ARUP Laboratories, Salt Lake City, UT, USA). After UIHC switched to the reverse algorithm, stand-alone RPR testing was available to order only as a follow-up test to check for treatment response [termed “Syphilis treatment follow-up (RPR with reflex titer)” in the electronic order entry system], as the reflex confirmation following an equivocal or positive syphilis IgG result, or for specific protocols (e.g., some organ transplant donor evaluations) that do not allow the use of reverse algorithm. Screening recommendations for syphilis at UIHC did not change during the retrospective time periods. For obstetric patients, the goal was universal testing unless specifically refused by the mother.

2.3. Retrospective chart review

Clinical histories were reviewed for patients who screened positive or equivocal for screening tests in either algorithm. Chart review included prior documented history of syphilis, prior treatment for syphilis, and treatment following results. Screening values/

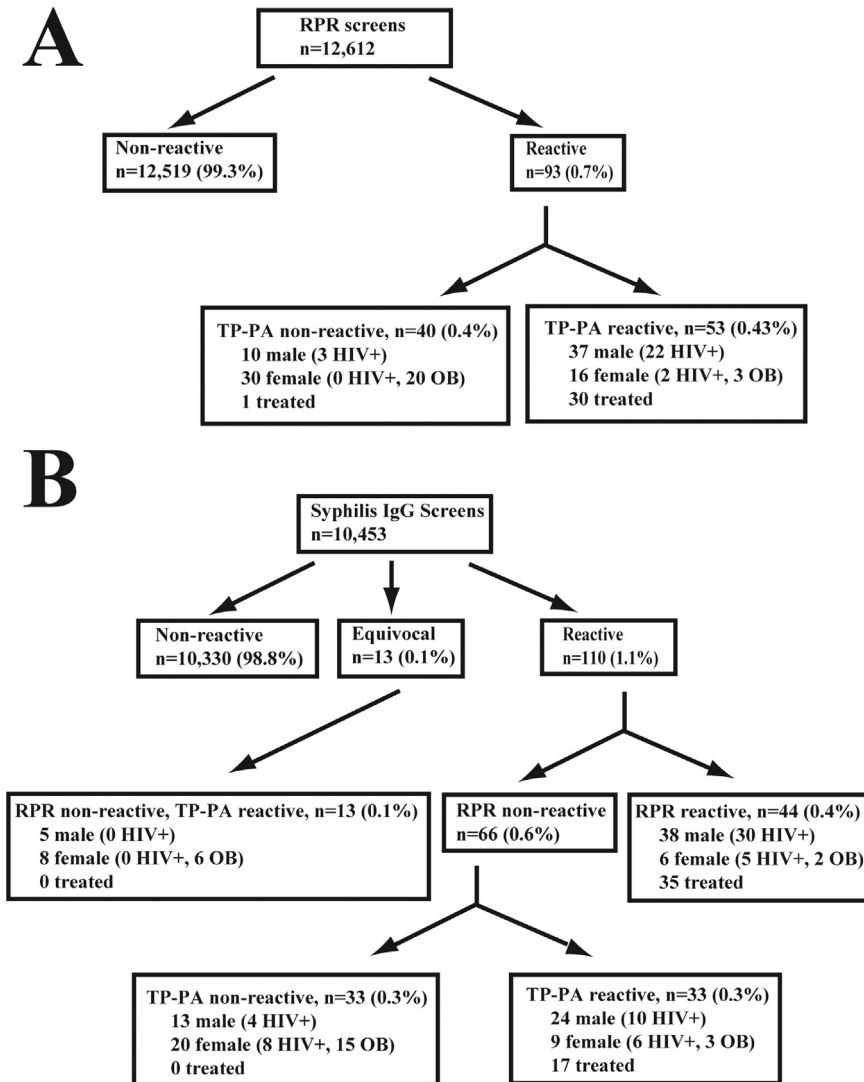


Fig. 1. Flow chart of patient results for the traditional (A) and reverse (B) syphilis testing algorithms. Percentages represent the percent of the total number of screening tests in the traditional and reverse algorithms. Abbreviations: HIV +, HIV-positive patient; OB, obstetric patient.

titers for the traditional and reverse algorithms (RPR and syphilis IgG) were also compared with confirmation status.

2.4. Statistical analysis

Statistical analyses were carried out in SPSS (PASW Statistics 18, Chicago, IL). Differences between the traditional and reverse algorithms with respect to total positive, positive, and false positive rates were determined by Chi-square analysis.

3. Results

3.1. Demographic breakdown before and after switch in syphilis algorithms

The retrospective analysis included 1396 days (3.8 years) in which the traditional syphilis algorithm was used (n = 12,612 RPR screens; average of 9.2 screens/day). During this time period, the average age of screened patients was 35.6 years (SD 14.1 years), and the male to female ratio was 0.35. The obstetrics (OB) department accounted for 47.8% of the total screens (a reflection of the goal for universal screening in this population) followed by non-OB outpatient/outreach clinics (41.3%), inpatient units (9.9%), and emergency department (1.0%). The demographics are summarized in Table 1.

The retrospective analysis included 959 days (2.8 years) in which the reverse syphilis algorithm was used (n = 10,453 syphilis IgG screens; average of 12.5 screens/day). During this time period, the average age of screened patients was 35.6 years (SD 13.4 years), and the male to female ratio was 0.37. The OB department accounted for 49.3% of screens followed by non-OB outpatient/outreach

Table 1

Demographics, timeframe, and patient location of patients tested by the traditional and reverse algorithms.

	Traditional algorithm	Reverse algorithm	P ^a
Total days of retrospective time period	1396	959	
Total screening tests performed (mean tests per day)	12,612 (9.2)	10,453 (12.5)	
Age in years (median, IQR)	31.5 (26.3–41.4)	31.8 (26.6–40.1)	
Age in years (mean, SD)	35.6 (14.1)	35.6 (13.4)	0.58
Males:females (ratio)	3201:9410 (0.35)	2822:7631 (0.37)	0.01
Patient location at time of testing			
Obstetric clinic or unit	6033 (47.8%)	5153 (49.3%)	0.03
Inpatient unit	1248 (9.9%)	911 (8.7%)	0.002
Non-obstetric outpatient/outreach clinic	5206 (41.3%)	4305 (41.2%)	0.90
Emergency department	125 (1.0%)	84 (0.8%)	0.15

IQR, interquartile range.

^a Paired *t*-test for age and chi-square analysis for other variables.

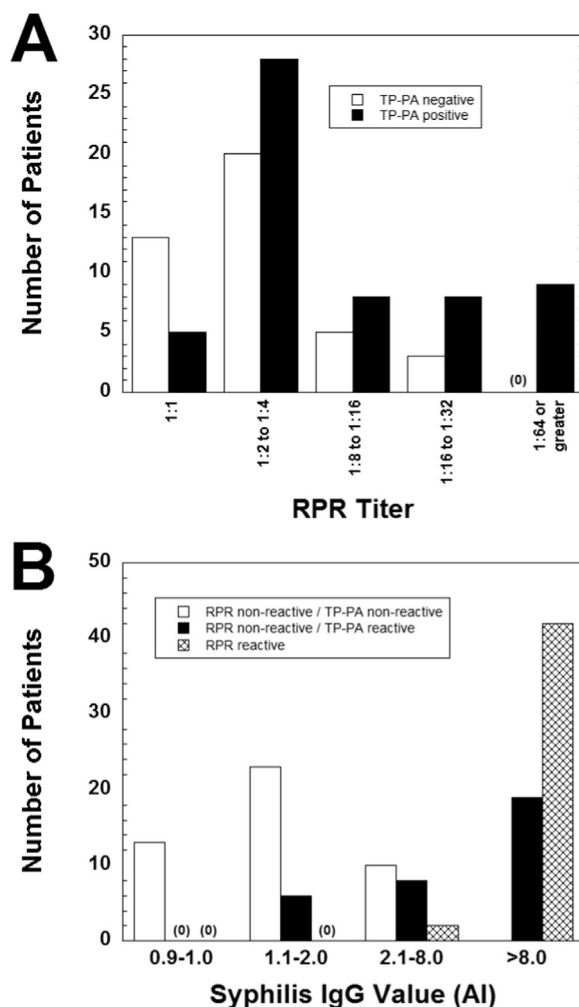


Fig. 2. Confirmation sorted by value of initial screening test. (A) For the traditional algorithm, the percent of samples that confirmed positive by TP-PA testing is sorted by whether initial RPR screen was 1:1, 1:2 to 1:4, 1:8 to 1:16, 1:32 to 1:64, or 1:128 or higher in titer. The percent of positive confirmations increases with RPR titer. (B) For the reverse algorithm, the percent of samples that confirmed positive by RPR or TP-PA testing is sorted by whether initial syphilis IgG screen was 0.9–1.0 AI (equivocal), 1.1–2.0 AI, 2.1–8.0 AI, or greater than 8.0 AI. The percent of positive confirmations increases with syphilis IgG value.

clinics (41.2%), inpatient units (8.7%), and emergency room department (0.8%).

3.2. Traditional syphilis algorithm results

A total of 12,612 screening RPR tests were performed as part of the traditional syphilis algorithm. Of those, 93 patients (0.7% of total) had a reactive RPR, with 53 patients (0.4% of total) confirming positive with TP-PA (i.e., RPR reactive, TP-PA reactive). Of the patients who confirmed positive, 29 had no documentation of prior syphilis, and of these, 25 had documented treatment after the result. The other 24 patients had documentation of prior treated syphilis; only five of these patients were treated after the result. Forty patients (43% of positive screen, 0.3% of total) did not confirm positive with TP-PA (i.e., RPR reactive, TP-PA non-reactive); none of these patients had a previous diagnosis of syphilis and only one was treated due to a highly suspicious lesion on his penis.

Fig. 1A summarizes the breakdown of patients who were reactive for the RPR screen. Of the 40 patients who screened positive but did not confirm in the traditional algorithm (i.e., RPR reactive, TP-PA non-reactive), 20 came from the OB clinic (50%) and 10 were male (male:female ratio = 0.33). Of the 53 patients who screened and confirmed positive in the traditional algorithm (i.e., RPR and TP-PA both reactive), 3 patients came from the OB clinic (6%), 37 were male (male:female ratio = 2.3), and 24 were HIV-positive (45%).

Fig. 2A shows how the RPR titer related to positive confirmation by TP-PA testing. Of patients who screened positive with a weak RPR titer of only 1:1 (n = 18), 13 were TP-PA non-reactive and 5 were TP-PA reactive (28% confirmation rate). Of patients who screened positive with an RPR titer of 1:2 to 1:32 (n = 66), 27 were TP-PA non-reactive and 39 were TP-PA reactive (59% confirmation rate). Of patients who screened positive with an RPR titer of 1:64 and above (n = 9), all confirmed positive with TP-PA testing (100% confirmation rate).

3.3. Reverse syphilis algorithm

A total of 10,453 screening syphilis IgG tests were performed following the switch to the reverse syphilis algorithm in February 2013. Of those, 13 patients (0.1% of total) screened equivocal with syphilis IgG values of 0.9–1.0 AI. Of these 13 patients, none had a previous diagnosis of syphilis, none confirmed positive (0% confirmation rate), and none were treated after the result.

There were 110 patients who screened positive (1.1 AI or higher) on the syphilis IgG assay. Of those who screened positive, 44 (40% of syphilis IgG positive, 0.4% of total) confirmed positive with a reactive RPR. Of these patients, 12 had a previous diagnosis of syphilis, with three in this group receiving documented treatment after the result. The other 32 positive syphilis IgG screens occurred in patients with no prior history of syphilis. All 32 of these patients had documented treatment after the result.

Of patients who screened positive with syphilis IgG values of 1.1 AI or higher, 33 were RPR non-reactive but TP-PA reactive (30% of the syphilis IgG positives, 0.3% of total). Ten of these patients had previous documentation of syphilis and of these 10, three received treatment after results. The other 23 had no previous diagnosis of syphilis, and 14 in this group received documented treatment.

Of patients who screened positive with syphilis IgG of 1.1 AI or higher, 33 did not confirm positive with either RPR or TP-PA (30% of syphilis IgG positive, 0.3% of total) and thus represent presumed syphilis IgG false positive screens. Within this group, only one had documentation of prior syphilis infection and treatment. None of these patients were treated after the result.

Fig. 1B summarizes the breakdown of patients who tested equivocal or positive for the syphilis IgG screen. Of the 13 patients who screened equivocal in the reverse algorithm, 6 came from the OB clinic (46%) and 5 were male (male:female ratio = 0.38). Of 33 patients who screened syphilis IgG positive with non-reactive RPR and TP-PA, 15 came from the OB clinic (45%) and 13 were male (male:female ratio = 0.59). Of the 33 patients who screened syphilis IgG positive and were RPR non-reactive but TP-PA reactive, 3 came from the OB clinic (9%), and 35 were HIV-positive (80%). 24 were male (male:female ratio = 2.67). Of the 44 patients who screened syphilis positive and confirmed with a reactive RPR, 2 came from the OB clinic (5%), 38 were male (male:female ratio = 6.33), and 16 were HIV-positive (48%) (Fig. 2A).

Fig. 2B shows the relationship of syphilis IgG antibody index with probability of confirmation. As noted above, none of the 13 patients with equivocal syphilis IgG screening results confirmed with either RPR or TP-PA. Of the patients who screened positive with a syphilis IgG value of 1.1–2.0 AI (n = 29), 6 confirmed positive with either RPR or TP-PA testing (21% confirmation rate). Of the patients who screened positive with a syphilis IgG value of 2.1–8.0 AI (n = 20), 10 confirmed positive (50% confirmation rate). Of the patients who screened positive with a syphilis IgG value of 8.1 AI or greater (n = 61; note that the assay reports these as > 8.0 AI and does not give an exact number at this higher range), all confirmed positive (100% confirmation rate).

3.4. Overall comparison between the traditional and reverse algorithms at UIHC

The reverse algorithm yielded significantly higher total screening positives (1.0% vs. 0.7%, $p = 0.01$, Chi-square analysis), true positive rates (0.7% vs. 0.4%, $p = 0.002$), and overall proportion of patients treated per patients screened (0.5% vs. 0.2%, $p = 0.002$). The false positive rates between the two algorithms (RPR reactive/TP-PA non-reactive for the traditional algorithm; syphilis IgG positive/RPR non-reactive/TP-PA non-reactive for the reverse algorithm) were not significantly different from one another (both 0.3%). If the 13 equivocal syphilis IgG screening results were included (all of which did not confirm), the total false positive rate for the reverse algorithm would be 0.4% and still not significantly different from the traditional algorithm ($p = 0.12$). The quantitative difference between the two algorithms is accounted for by patients who are syphilis IgG positive/RPR non-reactive/TP-PA reactive in the reverse algorithm.

4. Discussion

There is currently no gold standard test for *T. pallidum*, and it is not readily cultured or identified with simple laboratory stains [2,15], nor is darkfield microscopy commonly performed. Molecular tests for syphilis are mostly used in research and are not currently employed for routine clinical applications [1,15,16]. Serologic testing of treponemal and nontreponemal antibodies is the current standard for diagnosis. Treponemal and nontreponemal antibodies are detected starting approximately one week following infection (treponemal antibodies appear before nontreponemal antibodies) and may not be able to detect very recent acute infection [4,17]. In the past several years the CDC and Association of Public Health Laboratories proposed an update to replace the traditional syphilis algorithms with the reverse algorithm [3].

Nontreponemal screening, such as RPR in the traditional algorithm, is cost-effective and reliable in low prevalence settings and costs less in high prevalence settings [10]. RPR reactivity fluctuates with disease status and can be used to follow treatment efficacy and assess disease recurrence [1]. However, this method has disadvantages including decreased throughput, increased pipetting/manual labor, and subjective interpretation of results, [3] although there have been efforts to automate RPR testing [18]. Nontreponemal tests may show false positive screens due to a variety of reasons including lupus, viral mononucleosis, malaria, leprosy, viral pneumonia, and rickettsia infection [1].

Syphilis IgG assays can be done by high throughput, automated analyzers such as the Bio-Rad BioPlex 2200 instrument used in our institution. Treponemal screening may also be more sensitive than the traditional algorithm at detecting cases of secondary and late/latent syphilis [19]. However, one limitation of syphilis IgG assays is that past treated syphilis may still cause positive results for years; thus, syphilis IgG values are not useful on their own for following disease activity. Syphilis IgG assays are at least as sensitive as RPR with the exception of early primary syphilis (89%); if one excludes early syphilis, sensitivity approaches 100% [1,19].

In a geographic region of historically very low prevalence, the proportion of confirmed syphilis results increased from 0.4% to 0.7% following the switch from traditional to reverse algorithm (0.4% represents active disease only, while 0.7% represents active plus past treated). In our data, the difference between the two algorithms with respect to true positive rates is explained by the category of patients who are syphilis IgG positive / RPR non-reactive / TP-PA reactive who would be missed if RPR was the screening test. Our data showed equivalent clinical false positive rates between the two algorithms, which contrasts with some previous studies [4,7–10]. Because positive predictive value varies directly with prevalence, our reported positive predictive value is lower than that from published reports from locations with higher prevalence. Because prevalence of syphilis is so low in Iowa, our results likely approximate a lower bound on the positive predictive value of the reverse algorithm. Lastly, we noted an increased number of total screen positive patients (0.7–1.0%) in the reverse algorithm which is similar to other studies [7]. This study was performed at a large academic medical center with access to patient chart information to resolve unexpected results, and it important to note that this is not a direct comparison of the two algorithms.

The switch to the reverse algorithm has its drawbacks. There may be confusion and anxiety for providers and patients who are positive by treponemal tests but negative by nontreponemal tests [4,19]. It is important to resolve such discordant specimens by a second treponemal test [11]. The number of follow-up tests may increase with the reverse algorithm, especially if the population being tested has a high frequency of patients with past syphilis infection. There is also the possibility for over-treatment [10], which is especially a concern in the setting of medication shortages as occurred in 2016 with benzathine penicillin in the United States [20].

One major advantage of the reverse algorithm is automation of the screening assay. At UIHC, this reduced turnaround time for syphilis screening to less than two hours after receipt of specimen, which is considerably faster than the prior situation at our institution of batching RPR testing two to three times per week. With only a 1% positive screen rate, this means 99% of specimens rapidly return a negative result to the electronic medical record. The BioPlex analyzer also performs other assays at UIHC [14], allowing for much greater efficiency than maintaining RPR as a standalone, labor-intensive assay.

In the transition to the reverse algorithm at our institution, education was targeted towards clinical areas that frequently ordered syphilis testing, especially obstetrics/gynecology, infectious disease, and primary care outpatient clinics. All positive syphilis IgG screens are routed in the electronic medical record to a clinical pathologist for review, who then communicates with clinicians regarding interpretation and confirmatory testing. This helps in disseminating education, given that many clinicians at our institution encounter positive syphilis results infrequently due to overall low prevalence of syphilis in Iowa. We also encountered a number of challenges with ordering in the electronic medical record including: duplicate ordering of syphilis IgG and the syphilis treatment follow-up (RPR with titer) orders on the same specimen, provider orders for unnecessary additional confirmatory tests with a positive syphilis IgG screen (mostly caused by lack of understanding of the reflex reverse algorithm testing sequence), and repeat orders for syphilis IgG in known positive patients (in settings where RPR would be more appropriate to follow treatment efficacy). These types of issues required educational interventions and modifications in the electronic medical record such as duplicate cancellations and review/revision of electronic order sets containing syphilis testing [21].

At our institution, approximately half of the patients who are screened for syphilis were from the OB clinics, as part of an effort to universally test pregnant women. OB patients also accounted for approximately half of the false positives seen with either algorithm. In contrast, OB patients only accounted for 6% of the confirmed positives with either algorithm. Positive screening results for syphilis in pregnancy can result in considerable anxiety for providers and patients, given the potential risks of congenital syphilis. Additionally, the current shortage of benzathine penicillin demands a testing algorithm that generates as few false-positive results as possible to conserve doses for pregnant patients [20]. A prior report indicated that pregnant women with syphilis IgG positive/RPR non-reactive/TP-PA reactive results are at very low risk for adverse birth outcomes due to syphilis infection and do not require treatment [22]. In both algorithms at our institution, the majority of patients who confirmed positive were men (84% with traditional and 81% with reverse algorithm), which mirrors CDC data both nationally and within the state of Iowa showing an increase in

syphilis in men [12,13]. Although screening recommendations for syphilis at UIHC did not change during the retrospective time periods, there was a significant increase in the male:female ratio of those tested between the time periods in which the traditional and reverse algorithms were used. This may reflect factors such as increased clinician or patient awareness of high-risk factors for acquiring syphilis infection.

5. Conclusions

Our retrospective data add to the body of evidence that the screening titer for RPR and quantitative antibody index values for syphilis IgG correlate with the probability of confirmation [4,7,8,23,24]. The higher the RPR titer and syphilis IgG value, the more likely a patient is to confirm with subsequent testing. The association of high IgG strength with TP-PA activity has been previously reported, with some suggesting that an extra confirmatory TP-PA test is not needed with the reverse algorithm [24–26]. Education of providers using the reverse algorithm should include education that higher syphilis IgG values are likely to confirm, whereas equivocal or weakly positive values are less likely to do so. Similarly, for those still using the traditional syphilis algorithm, education can also reinforce that higher RPR titers are more likely to confirm that weaker titers. We found that increased detection of patients with syphilis IgG positive results correlated well with clinical diagnostic criteria, was relatively infrequent even in a low-prevalence area, and that these results were clinically actionable in the context of the entire reverse algorithm. Overall, the decision to use the traditional or reverse algorithm should be made based on a combination of the local syphilis prevalence, the expected workload, the requirement for automation, and the available budget [11].

Conflict of interest

None of the authors have any conflict to report.

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None

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