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Syphilis testing practices in the Americas

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Abstract

OBJECTIVE—To present the findings of the Pan American Health Organization’s 2014 survey on syphilis testing policies and practices in the Americas.

METHODS—Representatives of national/regional reference and large, lower-level laboratories from 35 member states were invited to participate. A semi-structured, electronically administered questionnaire collected data on syphilis tests, algorithms, equipment/commodities, challenges faced and basic quality assurance (QA) strategies employed (i.e. daily controls, standard operating procedures, technician training, participating in external QA programmes, on-site evaluations).

RESULTS—The 69 participating laboratories from 30 (86%) member states included 41 (59%) national/regional reference and 28 (41%) lower-level laboratories. Common syphilis tests conducted were the rapid plasma reagin (RPR) (62% of surveyed laboratories), venereal disease research laboratory (VDRL) (54%), fluorescent treponemal antibody absorption (FTA-ABS) (41%) and Treponema pallidum haemagglutination assay (TPHA) (32%). Only three facilities reported using direct detection methods, and 28 (41% overall, 32% of lower-level facilities) used rapid tests. Most laboratories (62%) used only traditional testing algorithms (non-treponemal screening and treponemal confirmatory testing); however, 12% used only a reverse sequence algorithm (treponemal test first), and 14% employed both algorithms. Another nine (12%) laboratories conducted only one type of serologic test. Although most reference (97%) and lower-level (89%) laboratories used at least one QA strategy, only 16% reported using all five basic strategies. Commonly reported challenges were stock-outs of essential reagents or commodities (46%), limited staff training (73%) and insufficient equipment (39%).

CONCLUSIONS—Many reference and clinical laboratories in the Americas face challenges in conducting appropriate syphilis testing and in ensuring quality of testing.

Keywords
syphilis testing; Pan American Health Organization; Americas; laboratory quality assurance; congenital Syphilis; elimination of mother-to-child transmission of syphilis and HIV

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Introduction

Each year, six million new cases of syphilis occur globally, most in low- and middle-income countries [1]. Syphilis infections are often asymptomatic; however, most adverse health outcomes can be prevented with early detection (screening tests) and prompt treatment with penicillin. This is particularly important in pregnancy, where syphilis screening and treatment are recognised as among the most highly cost-effective public health interventions available [2].

Untreated syphilis can cause damage to the central and peripheral nervous systems, cardiovascular system, liver, bones and joints. Primary infections can cause enhanced HIV acquisition and transmission. Syphilis in pregnancy can cause poor health outcomes in mothers and especially infants, as an estimated 50–80% of affected pregnancies (depending upon maternal stage) result in a serious, adverse foetal or infant outcome such as stillbirth, neonatal death, prematurity or congenital infection in the neonate [3]. In response to continued high numbers of untreated maternal syphilis infections globally, in 2007, the World Health Organization (WHO) launched a campaign for elimination of congenital syphilis. Despite substantial progress in the initiative, an estimated 350 000 adverse pregnancy outcomes, including 205 000 perinatal deaths caused by maternal syphilis, still occurred globally in 2012 [4].

In the region of the Americas, the point prevalence of syphilis in adults was estimated as 0.4% in 2012, representing about 937 000 new infections [1]. Most sexually acquired syphilis in Latin America and the Caribbean (LAC) occurred in individuals at high risk for HIV and other sexually transmitted infections (STI) (e.g. sex workers, migrant populations, men who have sex with men [MSM]) [5], with rising infections in MSM especially notable [6]. This situation is concerning as more syphilis infections, including HIV-syphilis coinfections, is likely to result in more cases of HIV through enhanced transmission. Additionally, higher syphilis prevalence in the general population translates into more syphilis cases among reproductive-aged women, leading to more congenital syphilis cases. In LAC, country-reported syphilis prevalence among pregnant women ranges from very low (0.1%) to very high (7.0%) [7]. In 2012, an estimated 63 000 infections during pregnancy contributed to 14 000 adverse perinatal outcomes in the region [3]. In 2015, PAHO estimated 22 800 cases of congenital syphilis occurred, a rate of 1.7 cases per 1000 live births [8]. Country-reported data from 2015 indicated that coverage of testing for syphilis in pregnant women was about 83% and treatment of women testing positive about 84%, with little improvement over the previous five years [8]. The stable, lower-than-recommended coverage occurred despite the Americas region focus on control and elimination of congenital syphilis and, since 2010, dual elimination of mother-to-child transmission of HIV and congenital syphilis (EMTCT) using integrated programmatic approaches [9].

Quality of syphilis diagnostic testing is a critical component of effective STI control programmes, including the regional initiative on EMTCT. The fact that most syphilis infections are asymptomatic limits use of direct detection approaches, thus laboratory testing relies primarily upon serologic tests – traditionally a non-treponemal screening test and treponemal confirmatory test. This testing strategy can prove difficult in settings with limited
or no access to laboratory-based services. Attempts at improving access to syphilis testing have identified potential efficiencies in using different algorithms based on laboratory availability and clinical situation [10]. For example, use of point-of-care (POC) diagnostics such as a rapid syphilis (treponemal) test (RST) has an advantage in settings where prompt treatment and low loss to follow-up are priorities (e.g. antenatal care). Regardless of the test types used and algorithms selected, implementing routine, internal and external quality assurance (QA) and quality control (QC) procedures are important to help ensure accurate testing and reduce the risk of misdiagnosis.

In 2014, PAHO conducted a regional survey to describe syphilis testing practices and the quality of testing among laboratories in the Americas. No previous survey of syphilis testing practices in the entire region had been previously attempted.

Methods

Survey participants were identified by technical advisors serving in the 35 PAHO member countries. In each country, the advisor was asked to identify the director or manager in charge of every national or regional reference laboratory and any large clinical laboratories (public or private) conducting syphilis testing. Identified contacts were invited by email to participate in the electronically administered survey through an online web link open between March and August, 2014.

The survey instrument was designed by a collaborative technical team from the PAHO, Washington DC office and the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta. The intent was to understand practices conducted in both reference laboratories and lower-level laboratory settings providing clinical services around syphilis testing, such as STI clinics, primary care clinics, HIV clinics and antenatal care (ANC) clinics. The original questionnaire was pilot-tested by three laboratory directors or managers in charge of syphilis testing in two large countries in the region. Base on the pilot test, a final questionnaire consisting of 94 structured, semi-structured and open-ended questions was developed in English and translated into Spanish for the survey.

Details of questions are previously described, [11] and (briefly) included information on syphilis tests used and number of tests conducted, commodities and equipment, testing algorithms, information on the technicians performing syphilis tests and challenges faced by laboratories. The questionnaire also asked about basic quality control (QC) and QA procedures used by the laboratories, including use of daily controls, availability of standard operating procedures for specific tests, type and extent of technician training on specific tests, laboratory participation in an external QA programme and periodic on-site evaluation aimed at assessing quality of testing. The questionnaire also asked about challenges faced by each laboratory, including stock-outs of essential supplies, which was defined as ‘running out of necessary reagents, test kits or other supplies needed to perform syphilis testing within the last year.’

Data were analysed using SAS version 9.3 (Cary, NC, USA) and Microsoft Excel 2013. If there was confusion about responses to open-ended question, an attempt was made to
contact respondents and clarify specific aspects of their responses. Descriptive analyses were performed to determine proportions and percentages of responses overall and stratifying by subgroups. Most analyses included stratification by national or regional (i.e., reference) laboratories and lower-level (i.e., more clinically focused) laboratories. Additional analyses assessed responses by subregion with countries grouped in the manner of previous PAHO reports, including North America (excluding Mexico), Central America (including Mexico), the Caribbean nations (Haiti, Guyana, Cuba and 12 other island nations), the Andean nations and the Southern Cone nations. We calculated proportions, means and medians values of variables using SAS version 9.3 and reported ranges where applicable [11].

Results

Participation

The 69 participating laboratories, representing 30 of 35 (86%) PAHO member states, were from Central America (n = 22, 32%), the Caribbean (n = 15, 22%), the Andean (n = 16, 23%), Southern Cone nations (n = 14, 20%) and North America (n = 2, 3%) (Table 1) [12]. Of the 69 laboratories, 41 (59%) were national or regional reference facilities and 28 (41%) were lower-level facilities that primarily provided clinical diagnostic testing (25 public and three private). Nearly half of the respondents were laboratory managers (n = 31, 45%), with other respondents including laboratory technologists covering syphilis testing (n = 26, 38%) and program managers or coordinators and researchers (n = 12, 17%).

Syphilis tests used by laboratories in the Americas region

The survey results indicated that direct detection methods were rarely performed by laboratories, even reference laboratories, in LAC. Only two reference laboratories reported using dark-field microscopy, of which one also reported conducting polymerase chain reaction (PCR) for *T. pallidum*. One regional laboratory reported using the direct fluorescent antibody for *T. pallidum* (DFA-TP).

For serologic testing, the most common non-treponemal test used among the surveyed laboratories was the rapid plasma reagin (RPR) (43 laboratories, 62%), followed by the venereal disease research laboratory (VDRL) assay (37 laboratories, 54%) and the unheated serum reagin (USR) test (two laboratories, 3%) (Figure 1). No laboratory reported using the toluidine red unheated serum test (TRUST). The survey did not include specific questions on use of qualitative and quantitative non-treponemal assays (e.g., RPR titre). Commonly used treponemal tests were the fluorescent treponemal antibody absorption (FTA-ABS) (28 laboratories, 41%) and the *Treponema pallidum* haemagglutination assay (TPHA) (22 laboratories, 32%), the *Treponema pallidum* particle agglutination assay (TPPA) (13 laboratories, 19%) and an enzyme immunoassay (17 laboratories, 25%) (Figure 1). Most (n = 65, 94%) but not all of the 69 participating laboratories conducted at least one non-treponemal test, including 38 (93%) of the 41 national or regional (reference) laboratories and 27 (96%) of the 28 lower-level laboratories. Similarly, most (n = 56, 81%) but not all of the 69 laboratories conducted at least one treponemal test, including 35 (85%) reference laboratories and 21 (75%) lower-level laboratories. Twenty-eight (41%) laboratories representing 16 (53%) of the participating countries reported conducting a rapid treponemal
test at their facility, including 19 (46%) of the reference laboratories and nine (32%) lower-level facilities.

**Syphilis testing algorithms**

Testing algorithms reported by the 69 participating laboratories are shown in Figure 2. Some laboratories reported using more than one type of algorithm. The most commonly used type of testing algorithm was a traditional approach involving a non-treponemal screening test and a treponemal confirmatory test, reported by 53 (77%) laboratories. Reverse sequence testing (initial testing with a treponemal test) was conducted by 15 (22%) laboratories. Most laboratories (62%) used only a traditional testing algorithm, 12% used only a reverse sequence algorithm, and 14% employed both algorithms. Nine laboratories (seven reference laboratories and two lower-level laboratories) reported conducting only one type of syphilis test (i.e. no algorithm). These included seven laboratories that used only an RPR or VDRL, one that used only a laboratory-based treponemal test and one that used only a rapid syphilis test.

Of laboratories using a traditional approach, most \((n = 33, 48\%)\) performed a non-treponemal screening test with reactive tests confirmed by a laboratory-based screening test (Figure 2). An additional nine laboratories (13%) used a non-treponemal screening test with reactive tests confirmed by a rapid treponemal test. Eleven laboratories (16%) performed initial non-treponemal tests with all tests confirmed by a laboratory-based treponemal test.

Among the 15 laboratories conducting reverse sequence screening, seven initiated screening with a laboratory-based treponemal test (three laboratories used EIA, two used CIA, one used TPPA and one used FTA-Abs), of which six facilities (9%) tested reactive results with an RPR or VDRL and one facility (1%) tested all results with an RPR or VDRL. The remaining eight laboratories (12%) initiated screening with a rapid treponemal test confirmed by an RPR or VDRL (five reference and three lower-level laboratories) (Figure 2).

Forty-nine laboratories (71%) reported having a recommended national algorithm for syphilis testing in pregnant women, most commonly a traditional approach, reported by 40 facilities (82%). An additional seven facilities (17%) used a reverse screening algorithm, and two laboratories reported using more than one algorithm in ANC settings.

**Quality assurance for syphilis testing**

Overall, 65 (94%) laboratories, including 97% of national/regional laboratories and 89% of lower-level laboratories, reported using one or more standard QA procedure for syphilis testing (Table 2). Regarding internal QC, the national/regional reference laboratories reported written standard operating procedures were readily available on site more commonly than lower-level facilities (76% compared to 50%, respectively). Most (83%) but not all laboratories performed daily serologic testing using controls, including 85% of reference and 79% of lower-level laboratories. About half of the laboratories \((n = 35, 51\%)\) reported conducting routine, onsite observations of laboratory testing performed in their facilities. Seventy per cent of responding laboratories reported participation in an external QA programme, including 83% of the reference laboratories and 50% of district/lower-level
laboratories. Just over half (59%, \( n = 24 \)) of the 41 reference laboratories, representing 18 countries, reported having proficiency testing programs in place for their underlying laboratories. Among district/lower-level laboratories, 18% reported being part of a proficiency testing program (Table 2) [13, 14].

73% of the 69 participating laboratories reported their technicians were specifically trained on conducting non-treponemal tests; and 49% on laboratory-based treponemal tests. Four laboratories reported that their technicians had no access to training of any type. Twenty-eight (41%) surveyed laboratories reported a need for additional training such as correct performance of specific tests, interpretation of test results and clinical management of STI cases (Table 3). Considering the five basic QA procedures asked about in the survey (written standard operating procedures, use of daily controls, laboratory participation in an external QA programme, routine on-site observations of testing performed and at least baseline training of technicians on use of specific tests performed in that laboratory), only 11 (16%) laboratories reported using all five strategies.

### Challenges

Data about stock-outs of test kits and supplies were reported by all 69 participating laboratories. Of these, 32 (46%) reported experiencing one or more stock-outs of an essential commodity, during the 12 months preceding the survey. At least one stock-out of needed testing reagents was reported by 55% of laboratories performing the VDRL testing, 46% of laboratories performing an EIA and 30% of the laboratories conducting the RPR test. Additionally, 26% of laboratories conducting RPR tests reported at least one stock-out of RPR cards, and some laboratories reported reusing RPR cards. Other essential testing supplies frequently reported to be unavailable were pipette tips (14% of laboratories) and gloves (17%). Several laboratories reported difficulty procuring specialised slides needed for testing cerebrospinal fluid (CSF).

Regarding equipment, over a third (39%) of the 43 laboratories performing VDRL testing did not have the orbital rotator required for these tests. Eight (19%) laboratories reported using a lateral rotator that is not recommended [15, 16]. Of the 17 laboratories reporting rotator age, a third were more than 15 years old. Of 29 laboratories that had microscopes that could be used for direct detection tests, three of them were more than 25 years old. Five laboratories provided a long list of equipment needs.

### Conclusions

The results of this first-ever survey on syphilis laboratory testing practices in the LAC region found that while most countries conducted basic syphilis testing, the quality of tests and testing may be less than optimal. For several countries, modest changes in syphilis testing practices could lead to large improvements in quality and cost-effectiveness of syphilis testing.

The use of direct detection methods was limited among laboratories, even in reference laboratories. Most (77%) surveyed laboratories used algorithms employing both a non-treponemal test and treponemal test. However, 12% of participating laboratories, including...
several reference laboratories, used only one test, either a non-treponemal test only or a treponemal test only, which was also an unexpected finding. As syphilis is a common infection in all countries, it is important that national/regional reference laboratories and large clinical laboratories have access to appropriate diagnostics including both treponemal and quantitative non-treponemal testing (at least one test type for each) and, ideally, direct detection methods [17].

The survey results suggested that although most laboratories maintained older syphilis testing approaches, others had adopted newer strategies. For example, many laboratories continued to use the FTA-ABS although it is a more costly test and requires more experienced technologists than the simpler TPHA or TPPA. On the other hand, just under 20% of laboratories had adopted reverse syphilis testing algorithms using automated or semi-automated immunoassays which reduce time and hazard for technologists, although are more costly than older serologic tests. Additionally, although RSTs have been widely available for almost two decades, the survey results found less than half of surveyed laboratories used these tests and, surprisingly, RSTs were more commonly used by reference laboratories than underlying clinical laboratories. Rapid point-of-care testing is a strategy that could be useful at any level, but because RSTs are more costly than laboratory-based tests in most countries, they are likely most cost-effective in clinical settings in which rapid return of results is important to ensure prompt treatment (e.g. antenatal clinics). RSTs may also be advantageous in improving coverage of syphilis testing in facilities which require patients to make an additional trip to the laboratory, as this additional step is not always made by patients for various reasons. In addition, the introduction of RSTs can be used as an opportunity to strengthen existing laboratory systems, and even ANC management, through enhanced training, stock management and quality assurance strategies [18, 19].

Another surprising finding was that basic practices to ensure quality of syphilis testing were often not performed. Importantly, many laboratories did not employ standard procedures such as use of daily controls or routine calibration of equipment. Many used inadequate rotators for serologic testing. An important component of quality assurance is adequately trained technicians [13, 14], but lack of opportunities for staff training was commonly reported by laboratories.

Assurance of accurate testing, whether laboratory-based or rapid testing, can be accomplished through participation in a proficiency testing program. For reference laboratories, for which there is an implied responsibility of ensuring quality of testing in underlying laboratories, participation in external QA is especially important. Quality of syphilis testing in peripheral laboratories can be achieved using a multipronged approach including development of a plan describing roles of reference and other laboratories, means of ensuring adequate training of staff, on-site standard operating procedures for all tests conducted and ongoing QA systems. The latter would include periodic site visits and observation (ideally as part of an over-arching laboratory QA programme) and reporting back from peripheral laboratories. The survey found that only about half of reference laboratories were providing reference capacity for syphilis testing to underlying laboratories, representing a missed opportunity for ensuring quality syphilis testing in many countries. On the other hand, at least one country (Brazil) had established a pragmatic national testing...
programme using simple dried tube specimens that could be reconstituted to allow underlying laboratories (or clinicians using rapid tests) to evaluate performance of both syphilis and HIV testing on the same sample. This low cost model based on procedures developed for HIV testing programmes is one that could be adopted by other countries [20, 21].

Many laboratories faced challenges in ensuring adequate supplies to carry out syphilis testing. Certain essential commodities such as adequate reagents that are not expired and specialised supplies (e.g. CSF slides, new RPR cards) are required for quality-assured syphilis testing. These would ideally be included as part of essential commodities projections and purchases. It is important that managers or logistic staff in charge of procurement understand the appropriate reagents and equipment required for accurate testing, and adequately forecast needs [22]. However, during a PAHO-sponsored meeting of laboratory directors from Latin America and the Caribbean, it was identified that laboratory personnel often lack the authority to purchase the correct types of equipment, commodities or both. This problem may require systematic level intervention that might be facilitated through collaborative work between health ministries and PAHO [23]. This approach may also help procure test kits, reagents and commodities at a more affordable bulk rate for the region.

There are some limitations in the survey. We sought to evaluate all national/regional reference and large clinical laboratories, but are uncertain of country-specific response rates or whether participating laboratories are representative of laboratories in the region. Private laboratories were particularly underrepresented. Many of the reporting laboratories were national or regional reference laboratories, and their responses may not reflect the experience of laboratories at lower-level health facilities. Not all member states participated, and data were particularly limited from small Caribbean island states. Data were largely based on the reports by directors and managers and may not reflect actual practice. Use of the electronic survey may have led to more misinterpretation of questions than would an in-person interview. Some critical questions were not included (e.g. use of quantitative and qualitative non-treponemal tests).

Syphilis in LAC continues to cause substantial morbidity and mortality in vulnerable populations including MSM, refuges and other mobile populations, pregnant women and infants. Sufficient quality of syphilis testing is important to adequately address this public health need. These survey results indicate that to ensure accurate syphilis diagnoses, more work is required to ensure quality of syphilis testing and availability of adequate supplies and equipment. The results provide some first steps towards supporting the Americas region in improving STI laboratory quality.

Acknowledgements

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References


Figure 1.
Syphilis serology tests reportedly used among laboratories in the Americas regions (N = 69)*. *Multiple choices possible, thus total does not add to 100%.
Figure 2.
Syphilis testing algorithms reported by participating laboratories (N = 69)*. *Multiple choices possible, thus total does not add to 100%.
### Table 1

| Participating countries by region (number of countries) [number of laboratories] |
|---|---|---|---|---|
| **North America**  
(N = 2) [N = 2] | **Central America**  
(N = 8) [N = 22] | **Caribbean**  
(N = 10) [N = 15] | **Andean**  
(N = 5) [N = 16] | **Southern Cone**  
(N = 5) [N = 14] |
| Nicaragua [1] | | | |
| | | | Saint Lucia [2] | |
| | | | Trinidad and Tobago [2] | |
### Table 2

Commonly used quality assurance strategies reported by participating laboratories ($N = 69$)

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Overall ($N = 69$)</th>
<th>National/regional ($N = 41$)</th>
<th>District/lower* ($N = 28$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Written standard operating procedures on site</td>
<td>45 (65)</td>
<td>31 (76)</td>
<td>14 (50)</td>
</tr>
<tr>
<td>Daily testing using controls</td>
<td>57 (83)</td>
<td>35 (85)</td>
<td>22 (79)</td>
</tr>
<tr>
<td>Routine, periodic observation of staff performing testing</td>
<td>35 (51)</td>
<td>22 (54)</td>
<td>13 (46)</td>
</tr>
<tr>
<td>Routine checks/maintenance of equipment</td>
<td>44 (64)</td>
<td>29 (71)</td>
<td>15 (54)</td>
</tr>
<tr>
<td>Routine procurement of reagents and/or test kits</td>
<td>37 (54)</td>
<td>22 (52)</td>
<td>15 (56)</td>
</tr>
<tr>
<td>Use of proficiency testing panels</td>
<td>29 (42)</td>
<td>24 (59)</td>
<td>5 (18)</td>
</tr>
<tr>
<td>Dried tube specimens to assess rapid treponemal tests</td>
<td>2 (3)</td>
<td>1 (2)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Participate in external quality control program</td>
<td>48 (70)</td>
<td>34 (83)</td>
<td>14 (50)</td>
</tr>
<tr>
<td>No quality assurance/QC strategies used at our facility</td>
<td>1 (1)</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* District/lower laboratories include district hospital laboratories, health care clinic laboratories and hospital and university laboratories.
Table 3

Proportion of laboratories with access to training for specific syphilis tests, by subregion (N = 69)

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Overall (N = 69)</th>
<th>North (N = 2)</th>
<th>Central (N = 22)</th>
<th>Caribbean (N = 15)</th>
<th>Andean (N = 16)</th>
<th>Southern cone (N = 14)</th>
<th>National/regional (N = 41)</th>
<th>District/lower (N = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory-based non-treponemal tests (RPR, VDRL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory-based treponemal tests (TPPA, TPHA, FTA-ABS, EIA, CIA)</td>
<td>51 (73)</td>
<td>1 (50)</td>
<td>17 (77)</td>
<td>14 (93)</td>
<td>10 (63)</td>
<td>9 (64)</td>
<td>30 (73)</td>
<td>21 (78)</td>
</tr>
<tr>
<td>Rapid treponemal tests</td>
<td>34 (49)</td>
<td>1 (50)</td>
<td>11 (50)</td>
<td>8 (53)</td>
<td>7 (44)</td>
<td>7 (50)</td>
<td>22 (54)</td>
<td>12 (44)</td>
</tr>
<tr>
<td>Use of Standard operating procedures, including maintenance of rotator and shaker</td>
<td>32 (46)</td>
<td>1 (50)</td>
<td>11 (50)</td>
<td>10 (67)</td>
<td>5 (31)</td>
<td>5 (36)</td>
<td>22 (54)</td>
<td>9 (33)</td>
</tr>
<tr>
<td>No access to training models/materials of any type</td>
<td>4 (6)</td>
<td>1 (50)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (19)</td>
<td>0 (0)</td>
<td>4 (10)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>