

Health Protection Agency

Standardisation of Virology Methods

STANDARD METHODS WORKING GROUP

Review of comments on the following SOPs:

VSOP 44 (review) – “*SEROLOGICAL DIAGNOSIS OF SYPHILIS*”

These comments were received by the “Virology” Working Group

Recommendations are listed as:

ACCEPT

REJECT

NONE

**PENDING
(review or evaluation)**

Issue date: 15.11.2007

Document

VSOP 44

Date Received

Jul 12,2007

Lab Name

Imperial College David Wright Emeritus Reader in Microbiology, Imperial College (Retired)

Section

Whole document

Comment

I have re-read the text of the consultation document and it still does not answer the principal objections. The Elisa may be easier to use with other automated tests but is unreliable compared with the standard TPPA[TPHA] and the now proposed discontinued FTA. This will lead to more sera being needlessly referred to the reference labs {I understand from my former colleague Dr Azadian at CHX that introduction of this test could lead to an extra 1000 referrals a year and this is one Unit alone!] This would perhaps be not so serious for smaller peripheral labs but is a dumbing down of the Service for the busier labs. What is perhaps more disturbing is the delay in diagnosing early syphilis while all these results are being investigated, which despite the prolonged incubation will increase the period for transmitting the disease!

Incidentally, the nature of the antigen used in this test has not been disclosed to me despite several letters to the company, one always feels uneasy using agents of unknown composition.

Recommended Action None see comment below

Thank you for taking the time to review VSOP 44 again. we did take on board your comments before and we would agree that the TPPA/TPHA test is more specific than the EIA resulting in less false positives and referrals.

However two points were considered in constructing the current algorithm:

The EIA is more sensitive (albeit less specific) and often comes up earlier in primary cases. It also is more sensitive than the TPPA in my experience in detecting past cases of syphilis. While you may not agree with it the strategy is, to use the most sensitive test, in this time of increasing numbers of syphilis

The second issue is that EIA, presumably for many reasons not least of ease of use, have become the screening test of choice and to recommend that TPPA becomes the screening test would mean a major upheaval of services. To my knowledge the Blood Transfusion centre have TPPA automated but there are very few other labs that do.

I share your concern about the delay in getting a report back to the patient and the philosophy behind writing this new SOP is to decrease turnaround time and get the patient's result out in

a timely manner. I am also working with the Regional Microbiology network to address this and it remains my highest priority.

Document

VSOP 44

Date Received

Jul 02,2007

Lab Name

Royal Cornwall Hospital

Section

Comment

While agreeing with the sentiment that syphilis is becoming more prevalent and minimising turnaround is important, I think the suggestion that RPR should be done at the primary lab is unwise. Referral laboratories have sufficient throughput to ensure consistent results for RPR and often deal with patients who have been seen at more than one centre. This is important because comparison of serial RPRs performed with the same reagents at the same laboratory is used as a measure of response to therapy or re-infection (BASSH guidelines).

Recommended Action Agree colour of box changed

Insisting that TPPA/TPHA is used as the local "confirmatory" test seems unnecessarily restrictive. We use an immunochromatic strip ("Assure" syphilis) the same day on any EIA positive samples because it is more suited to rapid one-off testing.

Recommended Action None insufficient evidence to recommend the change

Document

VSOP 44

Date Received

Jul 25,2007

Lab Name

HPA, Bristol Laboratory

Section

Comment

All cases should be tested with a total EIA (IgG/IgM). Any negative samples should be reported as 'No treponemal antibody detected. Please repeat if the patient is at risk of recent infection'.

Any EIA reactive sample should be tested by quantitative TPPA to 1280, RPR (screen at neat, and titrate to a minimum of 32 if positive), and IgM tested with results expressed as an antibody index if found positive (equal or greater than an index of 1.1).

If EIA total positive and TPPA negative and IgM negative the sample should be sent to a reference laboratory to resolve the anomaly (using a second line EIA or IgG immunoblot assay). If TPPA negative and IgM positive report as 'results suggest a very early treponemal infection. Please send a further sample to confirm.'

If EIA total positive and TPPA positive, report according to RPR and IgM results. If IgM is negative and the RPR is negative or less than a titre of 32, report as 'results consistent with treponemal infection at some time.'

If the RPR titre is 32 or greater and the IgM result is negative the report should indicate that 'recent treponemal infection cannot be excluded, treatment is indicated if not already given with repeat sampling advised in 3 months time'. Following the decline in RPR titre is useful as an indication of treatment success, conversely a rising RPR in a patient with previously positive treponemal serology with negative RPR results, may suggest treponemal re-infection.

If IgM result is positive above or equal to an Index of 2 with a negative RPR or at any RPR titre, report as 'Evidence of recent treponemal infection, treat (if not already treated) and repeat sampling in 3 months time.'

If IgM result is positive >1 but below an index of 2 and an RPR titre below 32 send to a reference laboratory to perform an IgM immunoblot and report as 'The IgM result may be consistent with treponemal infection in the recent past as IgM is known to persist for a period of 2 years at low titres. However, the IgM results may be non-specific, and treatment may not alter the serological status found.'

An IgM index >1-2 with an RPR of 32 or greater should be reported as 'consistent with treponemal infection in the recent past. Specific IgM is known to persist for a period of 2 years at low titre. If the RPR titre has not declined following treatment, allowing 3 months to monitor treatment response, re-treatment should be considered.'

The Bristol Laboratory has a significant experience of treponemal antibody testing. The following comments may be useful in developing the algorithm further.

1) If a primary laboratory is encouraged to do EIA (total) TPPA and RPR testing then it is within their powers to do specific IgM testing also. I would recommend that the issue of where a sample is tested is irrelevant to which tests are necessary to the flow chart of a 'diagnostic' evaluation. Therefore I advise removing the need for the coloured boxes. If a laboratory doesn't wish to do anymore than first line EIA testing there is no reason why another non-reference laboratory shouldn't do this for them together with an appropriate payment.

2) There is little point in doing a specific IgM test in an EIA (total) negative sample as even if the IgM was reactive it would have a very low index AI 0.9-1.1 are likely to be equivocal or indeterminate by repeat testing or IgM immunoblot, which doesn't make the result anymore definitive. It is more important to secure a repeat sample and initiate the treatment of symptomatic cases than the reporting of an equivocal/indeterminate specific IgM result, which will cause further delay in the reporting of the screen test.

3) The RPR and IgM should always be done on the first sample and not left to be 'considered' as a set of additional optional tests.

4) A quantitative TPPA is of limited value above a titre of 1280 in the first sample, and adds little to an interpretation of results that has evaluated both RPR and IgM results in follow-up.

samples. The value of the TPPA below 1280 is in its specificity rather than its quantitative sensitivity.

5) An EIA positive, TPPA negative status should be investigated in the first instance as a potential acute treponemal status and follow the same pathway as a EIA pos, TPPA pos result.

This then simplifies the algorithm.

Recommended Action None although these are valid points the algorithm is designed to be a minimum testing algorithm for Primary laboratories

Document

VSOP 44

Date Received

Nov 14,2006

Lab Name

Royal Infirmary of Edinburgh

Comments from Dr E Olson Microbiology Laboratory Royal Infirmary of Edinburgh

Section

Whole document

Comment

Comments on overall design of SOP.

The SOP does not explain the rationale behind the use of the investigations

Recommended Action Accept explanatory note written to accompany the algorithm.

There are several groups of patients investigated for Syphilis which require different strategies for investigation.

1. Low risk patients being screened for infection e.g. antenatal/ blood transfusion
2. High risk patients being screened for infection or a clinical diagnosis of syphilis e.g. contacts of syphilis or clinical suspicion of syphilis.
3. Patients with existing infection being staged and monitored for treatment response and reinfection.

Our approach is to use either an EIA for antenatal or a TPPA test for blood transfusion to screen low risk patients.

Recommended Action None they will then link in to the algorithm.

For patients in Group 2 we screen the patients with 4 tests EIA, TPPA, VDRL and IgM. I have noticed patients in this group with negative EIA results and would not endorse only using one test to screen these patients.

Recommended Action None this is adopted in the algorithm.

The current IgM assays are not completely reliable in diagnosing early syphilis and I would recommend using the VDRL as well an IgM.

Recommended Action None this is adopted in the algorithm.

I request repeat samples if all tests are negative at six weeks and three months if clinical suspicion of early syphilis

Recommended Action None this is a minimum testing algorithm.

For patients with existing infection we monitor all four tests. Some untreated/inadequately treated infections will be IgM negative.

Recommended Action None this is a minimum testing algorithm.

If the VDRL fails to fall fourfold by six months and 8 fold by one year post treatment we raise a concern about treatment failure or reinfection. If the VDRL or IgM titres rise significantly we raise a concern about reinfection. a persisting RPR titre of >16 is rarely seen in an adequately treated infection”.

We use the INNOLiA assay for difficult to interpret results.

Comments:

1. Line 5 implies no additional testing is required for known previous positives. As mentioned above this approach will miss treatment failures and reinfections. I would suggest deleting this line and box.

Recommended Action None it is not practical to separate repeat samples from new samples and clinical details are not always available to enable a distinction between the two to be made.

2. Line 6 Box that states “Consider RPR2,3, IgM EIA, quantitative TPPA/TPHA (if required). Suggest “Quantitative RPR2,3, IgM EIA, quantitative TPPA/TPHA (should be carried out in all cases. (I cannot think of an occasion when one would not do these tests on a new syphilis case.)

Recommended Action Accept algorithm amended.

3. Superscript comment 3 that currently reads “RPR titre is used in some laboratories to help assess whether infection is likely to be recent”. Suggest amend this to read “RPR titre is used in some laboratories to help assess whether infection is likely to be recent or adequately treated; a persisting RPR titre of >16 is rarely seen in an adequately treated infection. If the RPR fails to fall fourfold by six months and 8 fold by one year post treatment raise a concern about treatment failure or reinfection. If the RPR or IgM titres rise significantly raise a concern about reinfection”

Reason: Some untreated/inadequately treated infections will be IgM negative.

Recommended Action Accept algorithm amended.

4. Can I make a suggestion that investigation of neonates for congenital syphilis should be done by sending samples of blood from mother and baby to a reference laboratory and should be excluded from this protocol.

Recommended Action None the algorithm states that neonates and CSF samples are not covered in this VSOP.

5. Can you emphasis that all new patients should be confirmed on a second sample to avoid possibility of a sample labelling or handling error giving rise to a false result.

Recommended Action Accept will include this information in the accompanying guidance.

Looking at the protocol I would suggest that the minimum testing algorithm should be:

Low risk patients EIA or TPPA only

High risk patients EIA and TPPA. (EIA misses a small proportion of cases carrying out both tests will pick up more cases on first sample. This may be important if patient is not likely to come back for further testing.)

If a known contact of syphilis or primary syphilis suspected consider IgM and VDRL

Known infections: EIA TPPA VDRL and IgM

Recommended Action None EIA is less specific than TPPA

Document

VSOP 44

Date Received

Nov 14,2006

Lab Name

Section

Comment

1. Line 1 suggest IgG/IgM EIA rather than IgM/IgG EIA

Reason: the total antibody screening tests detect mainly IgG

Recommended Action Accept algorithm amended.

2. Line 4 comment under REPORT Box. Suggest "If a known contact of syphilis or primary syphilis suspected consider IgM"

Reason: as the comment stands "If patient symptomatic consider IgM" this would cover symptoms of secondary or late active syphilis. The non-reactive screening test should rule out these stages of infection – IgM testing would not be required (only time we are aware of a positive IgM as the only test positive is in very early primary infection).

Recommended Action Accept algorithm amended.

3. Line 6 Box that states "Consider RPR^{2,3}, IgM EIA, quantitative TPPA/TPHA (if required). Suggest "Consider quantitative RPR^{2,3}, IgM EIA, quantitative TPPA/TPHA (if required).

Reason: quantitative RPR is widely used and can be very helpful – the current comments denoted by superscript 2,3 support this.

Recommended Action Accept algorithm amended.

4. Line 7 first two boxes and corresponding reports. One box states "RPR^{2,3} Reactive IgM₄: Low positive" while the other box states "RPR^{2,3} Reactive IgM₄ : Positive".

Suggest remove the box and corresponding report that refers to Low positive IgM.

Reason: Low IgM is very subjective – what constitutes a low IgM? A "low" IgM might occur at the very early stage of an untreated infection or may be due to antibody persisting for up to a year after treatment. The existing report box "REPORT: Consistent with recent or active treponemal infection. Advise repeat if a newly diagnosed infection" remains relevant and can be retained.

Recommended Action Accept algorithm amended to include a subscript about low IgM.

5. Line 7 box that states "RPR² Reactive/Non-reactive IgM: Negative" Suggest "RPR^{2,3} Reactive/Non-reactive IgM: Negative" i.e. superscript³ has been added. Suggest the corresponding Report box that reads "REPORT: Consistent with treponemal infection at some time" is amended to "REPORT: Consistent with treponemal infection at some time. Advise repeat if a new diagnosis or at recent risk of re-infection."

Reason: All new diagnoses should be confirmed with a second specimen. Re-infection is relatively common in MSM at the present time.

Recommended Action Accept algorithm amended.

6. Superscript comment 3 that currently reads "RPR titre is used in some laboratories to help assess whether infection is likely to be recent". Suggest amend this to read "RPR titre is used in some laboratories to help assess whether infection is likely to be recent or adequately treated; a persisting RPR titre of >16 is rarely seen in an adequately treated infection".

Reason: Some untreated/inadequately treated infections will be IgM negative.

Recommended Action Accept algorithm amended.

7 General point: Should there be a note to state that although RPR has been used throughout this is interchangeable with VDRL carbon antigen test?

Recommended Action Accept to be added to accompanying guidance.

Document

VSOP 44

Date Received

Nov 06,2006

Lab Name

Imperial College David Wright Emeritus Reader in Microbiology, Imperial College (Retired)

Section

Comment

My comments on syphilitic serology are

1. If RPR and an ELISA/TPHA are done it will need a plasma and a sera to do a test. Plasma not usually obtainable.

Recommended Action Reject

2. Most modern ELISAs for detecting syphilis, do not state which molecular or otherwise devised antigens are used. and comparisons of different ELISAs may not be justified. They also give many more false positives than the TPHA

Recommended Action Reject

3. TPHA more reliable syphilis test. Only definitive report of false positives by Garner, are in leptotics from New Guinea

Recommended Action Reject

4. Why is more than one test for syphilis needed, especially if the TPHA or equivalent test is done according to makers directions, i.e. at dilutions of 1/80 and 1/160. One serological test is usually enough to diagnose most diseases. Automatic systems have been devised for this test.

Recommended Action Reject

5. Early diagnosis by use of an IgM component in an ELISAs are not usually very helpful, [and has a separate list of false positives] and in any event a true positive will be picked up in a further standard test.

Recommended Action Reject

6. I agree, the use of cardiolipin tests is a historical hangover and should be omitted [also the RPR] if reference lab confirmation is required then there are better tests FTA-ABS, TPI, immunoblots etc

Recommended Action Reject

Document

VSOP 44

Date Received

Oct 31,2006

Lab Name

Whipps cross microbiology

Section

Whole document

Comment

I understand how it has been designed. This is how we do it at Whipps Cross. We do a total (IgM & IgG) EIA screening test. Any reactives/equivocal we confirm with a second EIA test(different manufacturer)because we get a few BFP's plus an RPR test (including a titre). We then refer the test to reference laboratory (Colindale) who perform an IgM, TPPA, RPR and EIA. These tend to be reported with a titre. Any discrepancies we repeat all tests, if still disagreement we refer to reference laboratory.

Recommended Action Reject. In order to give a more timely result particularly in infectiousness it is our recommendation to replace the second EIA with a TPPA/IPR performed at primary diagnostic laboratory to allow a report to be issued. Any confirmation of discrepant results should go to the reference laboratory.

Document

VSOP 44

Date Received

Oct 16,2006

Lab Name

City Hospitals Sunderland NHS Trust - Microbiology

Section

Serological diagnosis of syphilis

Comment

Here in Sunderland we are still screening with a combination of RPR + TPPA but are planning to switch to the Abbott Architect IgM/IgG EIA,at which stage we will probably retain the ability of doing RPRs but not TPPAs (RPRs are easier to do on an occasional basis). VSOP 44 appear to suggest that any reactive EIA test should be confirmed by TPPA, regardless of whether it is a "first" positive or a "repeat" positive.

Recommended Action None the algorithm does not recommend distinguishing between first and repeat samples

I do not have a problem with the first positive having to be confirmed by TPPA (it needs to go to the reference laboratory anyway) but "repeat" positives do not really need confirmation by TPPA (or a reference laboratory)as the repeat positive EIA is all it is required to exclude a

possible sample mix up with the first specimen. On "repeat" positives we are planning to do a quantitative RPR (this is more useful than a quantitative TPPA to monitor response to treatment) but no TPPA at all. Should a separate algorithm be used for "repeat" positives?

Recommended Action Reject EIAs not totally specific and should be confirmed using an IPPR

Document

VSOP 44

Date Received

Oct 10,2006

Lab Name

Dundee

Section

Algorithm

Comment

Advice on the follow up testing of acute cases would be useful: when and how often should cases be tested.

Recommended Action Reject advice already available