Laboratory Approach in PMTCT HIV and Syphilis

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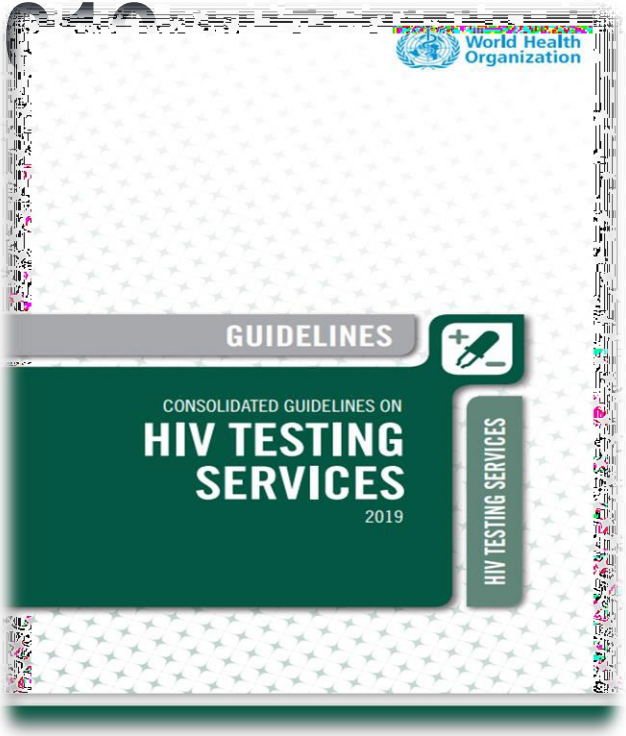
Prevention of Mother to Child Transmission (PMTCT) of HIV and Syphilis Course,
8th May 2024

Part 1: Screening and diagnostic testing of HIV

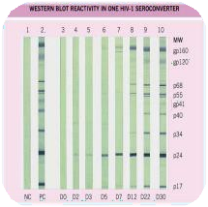
WHO Guidelines 2019



WHO has updated recommendation on HIVST and provides new recommendations on social network-based HIV testing approaches and western blotting

HIV Diagnosis and Testing Strategies

Recommendations and Good



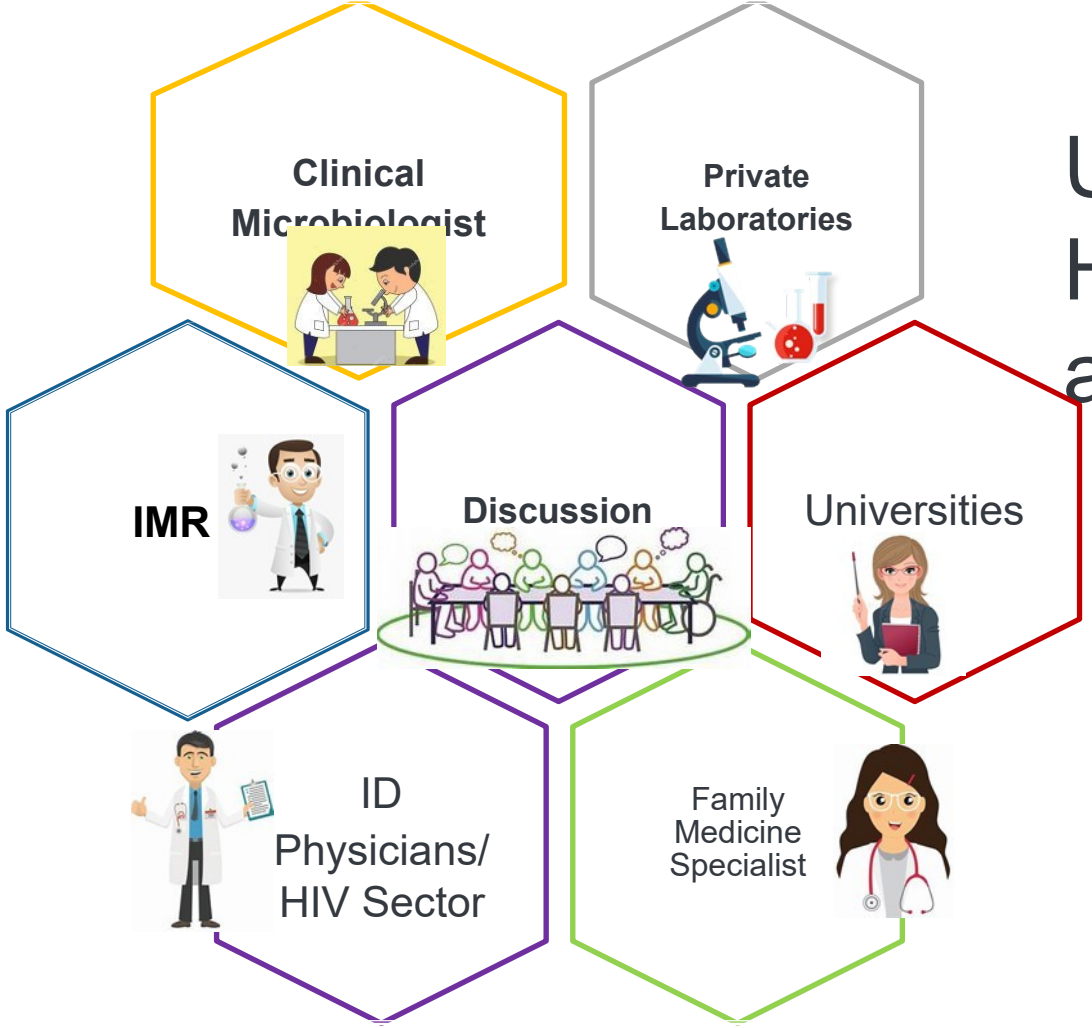
Western Blot and line immunoassay should not be used in the national testing algorithm

HIV testing algorithm to achieve 99% PPV

Use tests with ≥99% sensitivity and ≥98% specificity

First Test: Highly sensitive

Subsequent Test: More specific



Updating the HIV testing algorithm

- Through series of meetings since 2019
- IMR was appointed as the secretariat

HIV testing guideline was published on 4th December 2020

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SEPERTI SENARAI EDARAN

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SURAT PEKELILING KETUA PENGARAH KESIHATAN MALAYSIA BIL.10/2020

KEMASKINI CARTA ALIR UJIAN SARINGAN DAN PENGESAHAN HIV

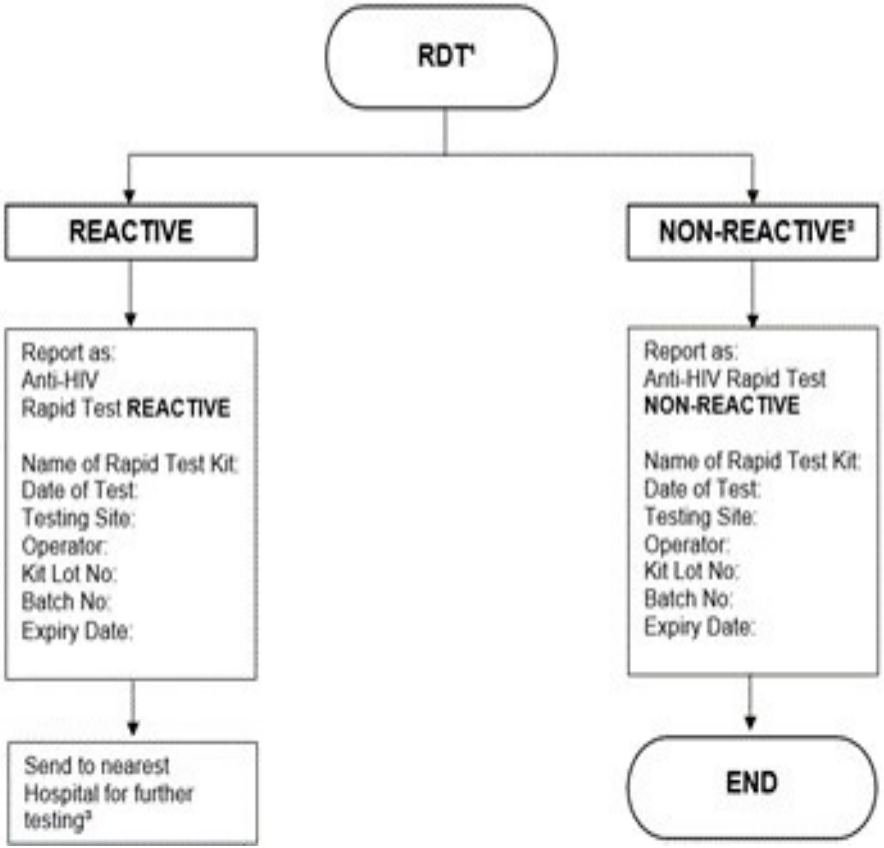
1. TUJUAN

Memaklumkan kemaskini carta alir ujian saringan dan pengesahan HIV sebagai panduan yang mesti diikuti dalam mengendalikan ujian saringan dan pengesahan HIV oleh anggota kesihatan di klinik-klinik kesihatan, hospital dan

HIV Testing Algorithms

1. **Rapid Diagnostic Test**
2. HIV Antigen/Antibody Combination Assay for Adult & Children > 18 months
3. HIV PCR for paediatric < 18 months

Algorithm 3: Algorithm for HIV Antibody Testing Using Single Rapid Diagnostic Test (1-RDT Strategy)



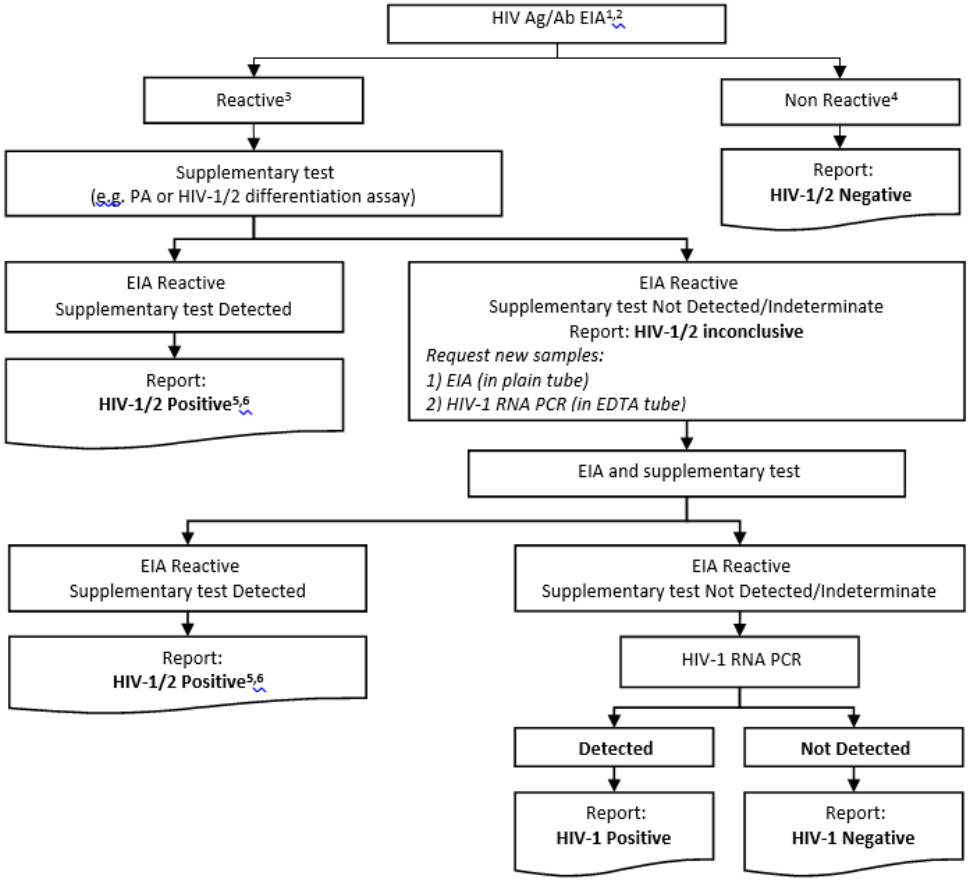
HIV-negative individuals with ongoing risk (for retesting after 4-6 weeks):

- ❑ people from key populations
- ❑ people with a known HIV-positive partner
- ❑ people with known recent HIV exposure individuals seen for a diagnosis or treatment of STIs
- ❑ TB patients with a possible recent HIV exposure or who are at higher risk for HIV exposure
- ❑ outpatients with clinical conditions indicative of HIV infection
- ❑ individuals taking PEP or PrEP
- ❑ pregnant and breastfeeding women in high incidence / prevalence settings*

***Note: retesting in third trimester and during postnatal**

New HIV Algorithm using HIV Antigen/Antibody Combination Assay

Algorithm 1: Algorithm for Laboratory HIV Testing Using HIV Antigen/Antibody Combination Immunoassay for Adults and Pediatrics Age Group > 18 months



[1] HIV Ag/Ab EIA: HIV antigen-antibody enzyme immunoassay (latest available version).

[2] Specimen is considered as first specimen if there is NO previous RDT result. Specimen is considered as second specimen if there is previous positive RDT result (first specimen).

[3] To be performed as duplicate or more following the manufacture product inserts.

[4] If there is possibility of very early infection leading to a non-reactive on the initial antigen/antibody immunoassay, such as when recent HIV exposure is suspected or reported, request for a new specimen and repeat the algorithm after 2-4 weeks or to conduct an HIV-1 RNA PCR.

[5] Request second sample for patient verification if no previous positive result documented. This is applied for EIA and supplementary testing only.

[6] Suggest: to send fresh blood sample in EDTA tubes for HIV-1 RNA PCR

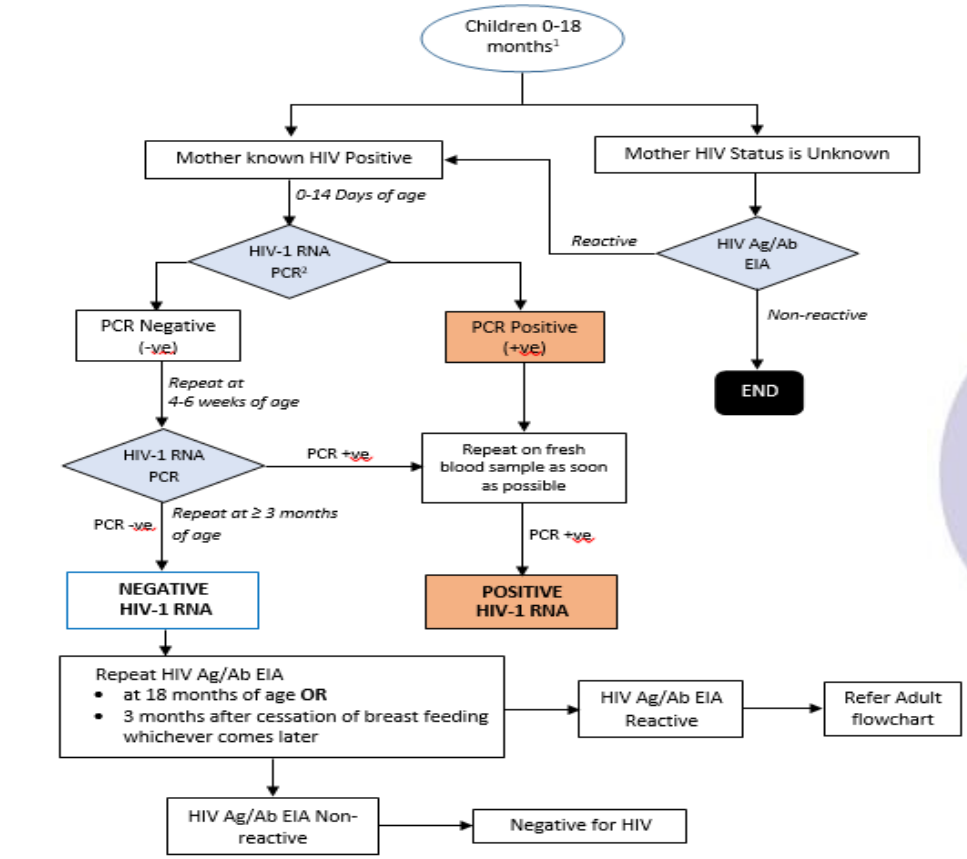
References:

1) *World Health Organization (WHO). Consolidated Guidelines on HIV Testing Services for A Changing Epidemic. Policy Brief. November 2019.*

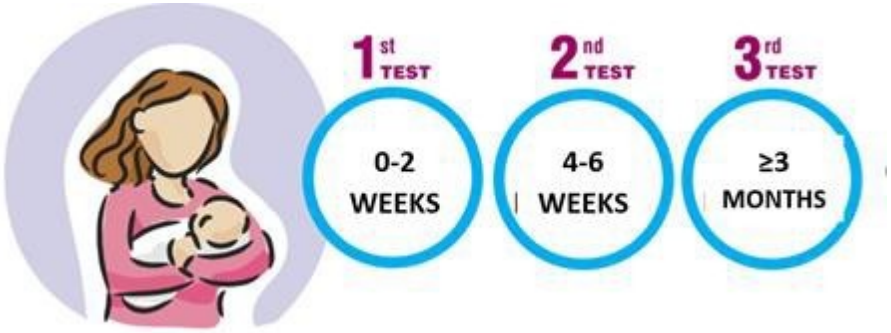
2) *Centers for Disease Control and Prevention (CDC). 2018 Quick Reference Guide: Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens. CDC website. https://stacks.cdc.gov/view/cdc/50872. Updated January 2018. Accessed 6 February 2020.*

HIV Testing Algorithm for Paediatrics < 18 months of age

Algorithm 2: Algorithm for Diagnosis of HIV Infection for Pediatrics Age Group < 18 months



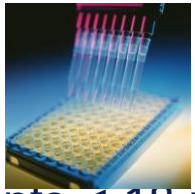
[1]Includes abandoned babies
[2]HIV RNA test: 2.5mls of blood in EDTA. Specimen need to be transported at ambient temperature as soon as possible to IMR KL (Virology). If unable to send within 24 hours, keep specimen at 2-8°C (maximum of 3 days).



Confirming HIV Test Results in Infants

At least **two HIV-1 RNA PCR** test results must be **negative** to be sure that a baby is not infected with HIV.

At least **two HIV-1 RNA PCR** test results must be **positive** to know for certain that a baby is infected with HIV.



In infants < 18 months of age with HIV-positive mother, where the test is **missed by the recommended age, HIV-1 RNA PCR test need to be done**

Rapid Diagnostic Test

- In Malaysia, HIV RTKs for primary care laboratories are procured centrally by the Ministry of Health and distributed to the respective states to be supplied to the relevant clinics.
- Sensitivity & Specificity: ≥ 99.0 & ≥98.0, respectively
- RTKs are sent by 4 consignments each year and with each consignment, the kit will be tested and evaluated for its performance

HIVST Guideline was published in August 2023

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SEPERTI SENARAI EDARAN

YBhg. Datuk /Dato' Indera /Datin Paduka /Dato' /Datin /Tuan /Puan,

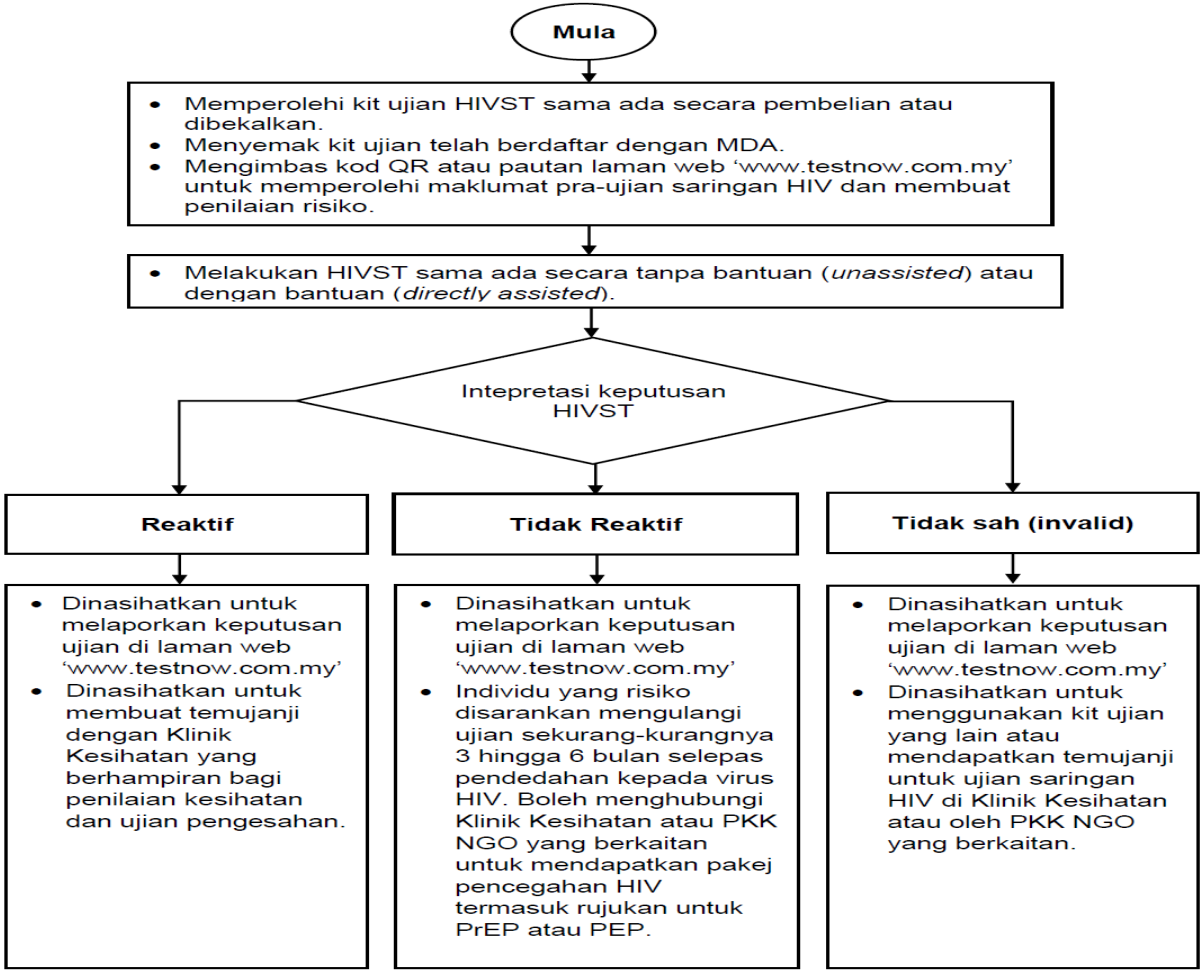
SURAT PEKELILING KETUA PENGARAH KESIHATAN MALAYSIA BIL. 14 /2023 : GARIS PANDUAN PELAKSANAAN UJIAN SARINGAN HIV KENDIRI DI MALAYSIA

1. TUJUAN

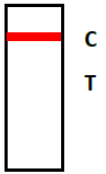
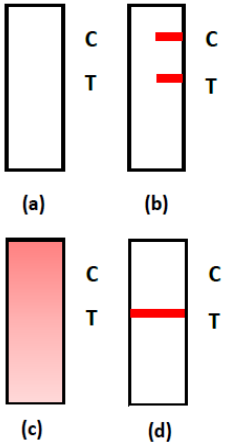
Surat Pekeliling Ketua Pengarah Kesihatan Malaysia Bil. 14 /2023 ini bertujuan untuk menerangkan dasar dan garis panduan pelaksanaan ujian saringan HIV kendiri di Malaysia.

Lampiran 1

Carta alir pelaksanaan ujian saringan HIVST



Jadual 1: Contoh-contoh interpretasi keputusan HIVST *rapid test*

	**Keputusan rapid test 'tidak reaktif':** - Satu garisan lengkap muncul di bahagian "C" dan tiada garisan muncul di bahagian "T".
	**Keputusan rapid test 'reaktif':** - Dua garisan yang lengkap muncul di bahagian "C" dan "T". - Jika garisan yang muncul di "T" tidak terang atau kabur, keputusan ujian masih dianggap reaktif.
	**Keputusan rapid test 'tidak sah (invalid)':** - Tiada garisan muncul di "C"; atau - garisan muncul hanya sebahagian; atau - latar belakang merah muncul menyebabkan keputusan tidak dapat dibaca; atau - hanya garisan "T" sahaja yang muncul

Jadual 2: Ringkasan kepada tindakan selepas intepretasi keputusan HIVST *rapid test*

Keputusan HIVST (kaedah *rapid test*)	**Risiko**	
	Ada	**Tiada**
Reaktif	Individu berkemungkinan telah dijangkiti virus HIV. Dinasihatkan membuat temujanji dengan Klinik Kesihatan yang berhampiran bagi penilaian kesihatan dan ujian pengesahan.	Individu berkemungkinan telah dijangkiti virus HIV. Dinasihatkan membuat temujanji dengan Klinik Kesihatan yang berhampiran bagi penilaian kesihatan dan ujian pengesahan.
Tidak reaktif	Individu berkemungkinan masih berada di dalam tempoh '*window period*'. Hentikan amalan yang berisiko dan mengulangi semula ujian sekurang-kurangnya 3 hingga 6 bulan selepas pendedahan kepada virus HIV. Boleh menghubungi Klinik Kesihatan atau PKK NGO yang berkaitan untuk mendapatkan pakej pencegahan HIV.	Individu tidak dijangkiti virus HIV. Boleh mengulangi ujian bila perlu.

Part 2: Screening and diagnostic testing of Syphilis

Syphilis - *Treponema pallidum*

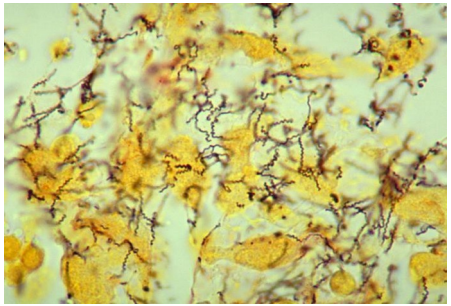


Image credit: CDC/Dr. Edwin P. Ewing, Jr. (PHIL #836), 1986.

- Spirochaete bacteria
- Difficult to culture and see under light microscope
- Laboratory diagnosis rely on serology testing using:

non-treponemal test (RPR and VDRL) **and**

treponemal test (TPPA/TPHA/rapid treponemal assays (RDT)/CIA/EIA/FTA-ABS

Laboratory investigations of syphilis

a. Non-treponemal

- **VDRL (Venereal Disease Research Laboratory) test**
- **RPR (Rapid Plasma Reagin) test**

- Detect antibody toward **reagin**, generated from interaction of the host with *T. pallidum*

- Not specific to *T. pallidum* infection

- cheap, easy to perform, fast,

Laboratory investigations of syphilis

- Non-treponemal titers correlate with disease activity
- are used to follow treatment response
- **titer decline with time after treatment**
- A fourfold change in titer or a change of two dilutions - significant difference e.g 1:16 to 1:4 or 1:8 to 1: 1:32,

Laboratory investigations of syphilis

- Repeated testing on same patient should be performed using same test and preferably by the same laboratory

- Titer from RPR test cannot be compared with VDRL directly because RPR titers are slightly higher than VDRL titers

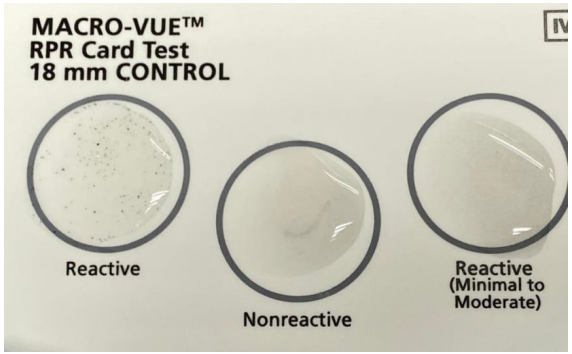
Laboratory investigations of syphilis

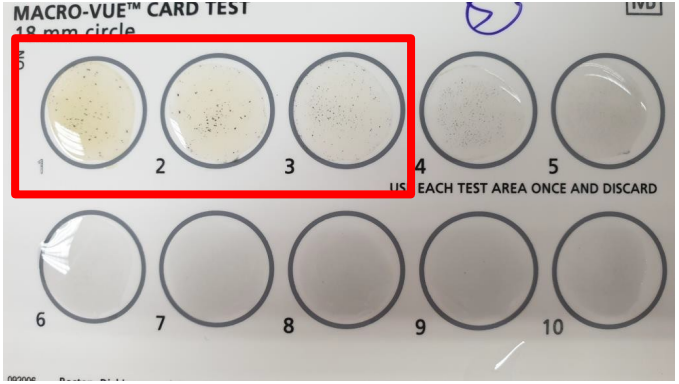
RPR vs VDRL

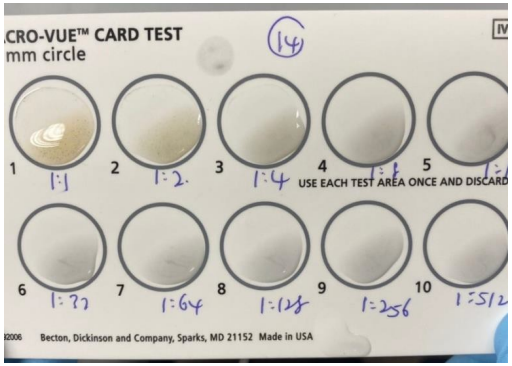
VDRL test – microflocculation assay,
need microscope to see the flocculation

RPR test – macroflocculation assay,
able to see flocculation without microscopy,
with addition of charcoal

Laboratory investigations of syphilis







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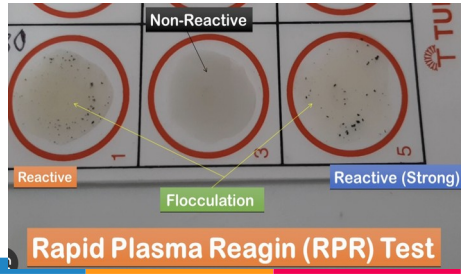
How to measure “fourfold” titre ?

A titer - is a measure of the amount of antibody formed in response to syphilis, Titer will be decline after proper treatment over a period of months to years
It is preferable to compare the same non-treponemal tests when determining a new infection or to verify adequate response to treatment on individual
No definitive cut-off titre can be used to determine the active infection

Decline at least fourfold indicates proper treatment

1:128
1:64
1:32
1:16
1:8
1:4
1:2
1:1

A fourfold increase/higher indication a new infection



Laboratory investigations of syphilis

False positive non-treponemal test

- Infectious causes – e.g Malaria, Tuberculosis, viral fevers
- Non-infectious causes - Drug addiction, connective tissue disease, pregnancy, older age

Positive non treponemal test need to be confirmed using treponemal test

Laboratory investigations of syphilis

b. Treponemal test

- detect antibodies specific to Treponema species
- remain positive for life in that previously had syphilis
- Cannot differentiate between previous or current infection

Laboratory investigations of syphilis

Treponemal test

- ***Treponema pallidum* Particle Agglutination (TPPA)**
- *Treponema pallidum* Haemagglutination (TPHA)
- **Rapid test**
- **Enzyme immunoassay**
- Fluorescent treponemal antibody adsorbed (FTA-Abs) test – not available in KKM lab

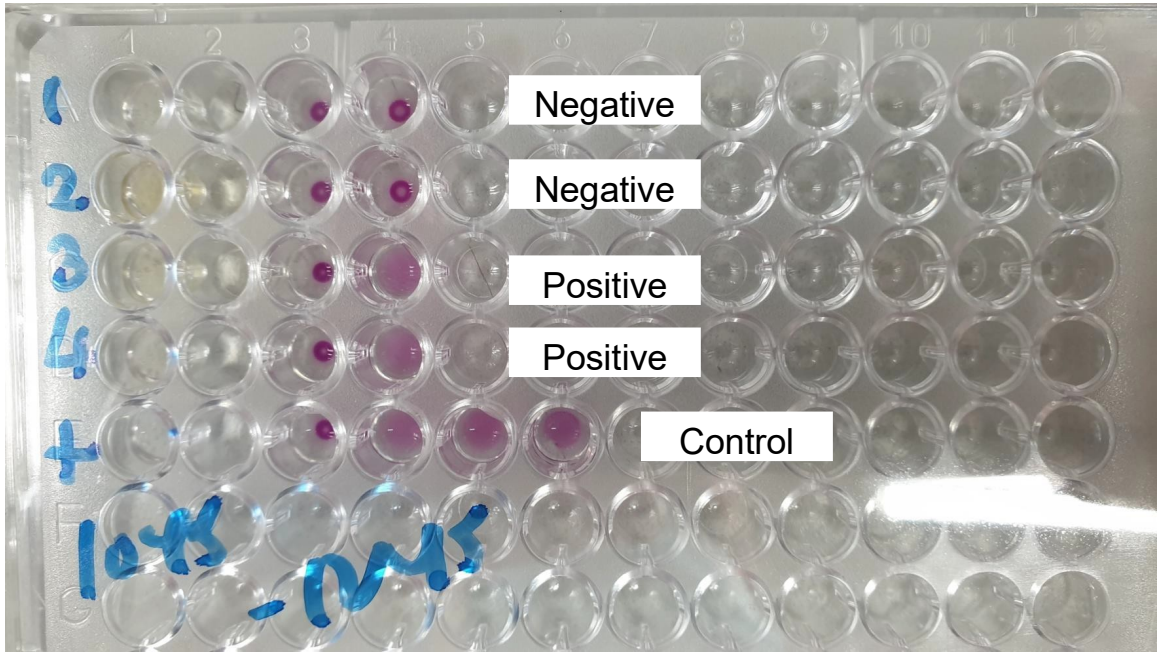
Laboratory investigations of syphilis

1. *Treponema pallidum* particle agglutination

- agglutination of gel particles sensitized with *T. pallidum* antigens by antibodies found in the patient's serum.
- 3 hours, usually done in batches
- **LTAT varies 1 day to 7 days**
- available in state and major specialist hospitals only.

Laboratory investigations of syphilis

Treponema pallidum particle agglutination



Laboratory investigations of syphilis

2. Treponemal immunoassay

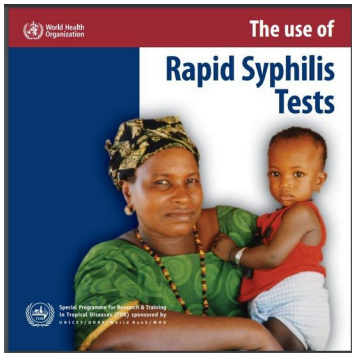
- enzyme immunoassay (EIA), chemiluminescence immunoassay (CIA), immunoblot

- Use in high-volume laboratories, reducing labor and turnaround time.

Laboratory investigations of syphilis

3. Rapid syphilis test

- simple point-of-care
- Card or strip
- easy to perform, minimal training
- no equipment needed
- use finger prick blood
- fast result - within 30 minutes
- Dual test kit is available (HIV/Syphilis)



Laboratory investigations of syphilis

Rapid syphilis test

- Sensitivity ranges from 85% - 98%
- Specificity ranges from 93% - 98%

  (WHO)
- Approved kits to be used in Malaysia is available on MDA website

Laboratory investigations of syphilis

Criteria for choosing RDT

- Test Performance
- Ease of use
- Conditions of use
- Conditions of storage
- Long shelf life

Laboratory investigations of syphilis

Other methods

1. **Dark field microscopy**

   detects Treponema pallidum based on morphology and motility

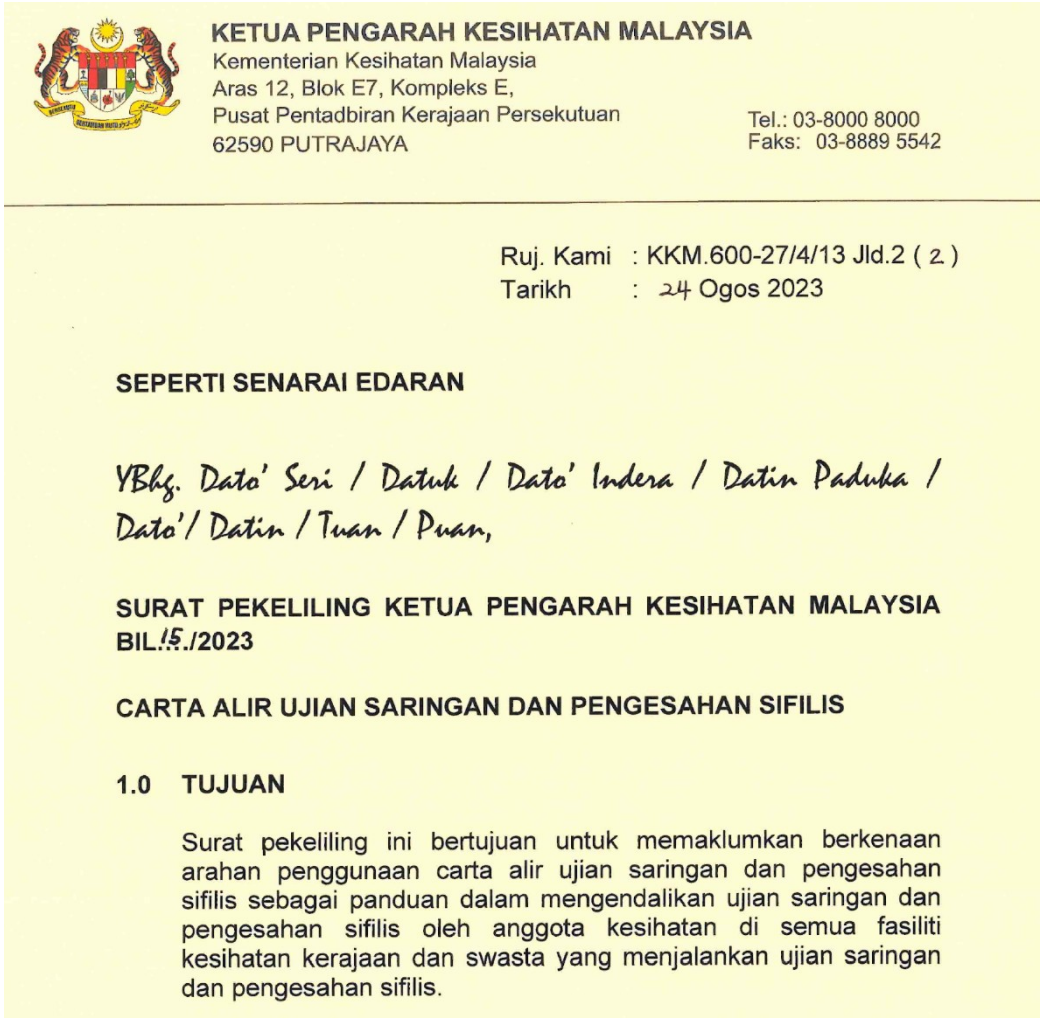
   need skill personnel

2. **Nucleic acid testing**

   Useful in primary syphilis in lesions,

   not useful for blood and serum

Latest syphilis guideline was published in August 2023



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Tarikh : 24 Ogos 2023

SEPERTI SENARAI EDARAN

YBhg. Dato' Seri / Datuk / Dato' Indera / Datin Paduka / Dato' / Datin / Tuan / Puan,

SURAT PEKELILING KETUA PENGARAH KESIHATAN MALAYSIA BIL.15./2023

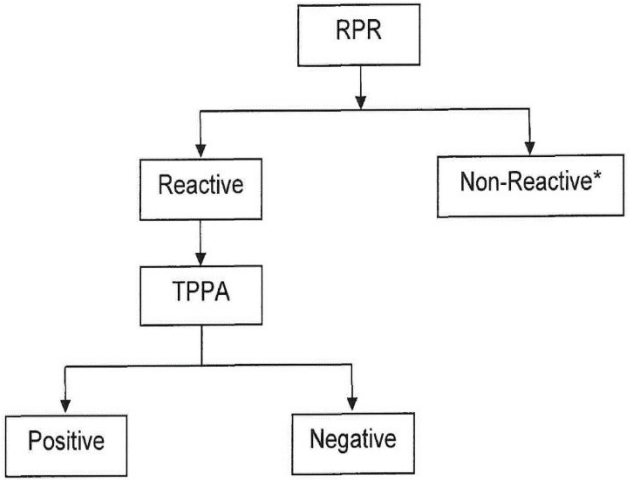
CARTA ALIR UJIAN SARINGAN DAN PENGESAHAN SIFILIS

1.0 TUJUAN

Surat pekeliling ini bertujuan untuk memaklumkan berkenaan arahan penggunaan carta alir ujian saringan dan pengesahan sifilis sebagai panduan dalam mengendalikan ujian saringan dan pengesahan sifilis oleh anggota kesihatan di semua fasiliti kesihatan kerajaan dan swasta yang menjalankan ujian saringan dan pengesahan sifilis.

LAMPIRAN 2

ALGORITHM 1: SYPHILIS TESTING USING 'TRADITIONAL ALGORITHM'



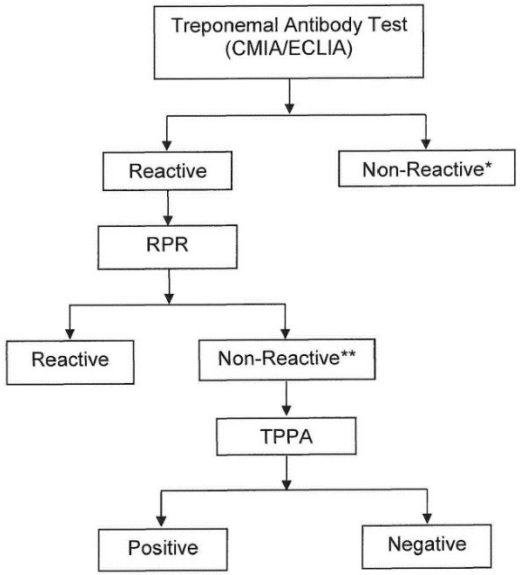
Note:
RPR = Rapid Plasma Reagin
TPPA = *Treponema pallidum* Particle Agglutination
*In the absence of high-risk behaviour/exposure, syphilis is unlikely. Please correlate with clinical findings.

LABORATORY INTERPRETATION

RPR	TPPA	Interpretation
Non-Reactive	N/A	In the absence of high-risk behaviour/ exposure, syphilis is unlikely. Please correlate with clinical findings. Suggest to repeat testing after 2-4 weeks if clinically indicated.
Reactive	Positive	Suggestive of syphilis infection (previously treated or untreated syphilis).
Reactive	Negative	Syphilis infection is unlikely; possible biological false positive. If at risk for syphilis, repeat the test in 2-4 weeks.

N/A = Not applicable

ALGORITHM 2: SYPHILIS TESTING USING 'REVERSE ALGORITHM'



Note:
CMIA = Chemiluminescence Immunoassay
ECLIA = Electrochemiluminescence Immunoassay
*In the absence of high-risk behaviour/exposure, syphilis is unlikely. Please correlate with clinical findings.
** Check for previous TPPA result. If TPPA is positive, no need to proceed.

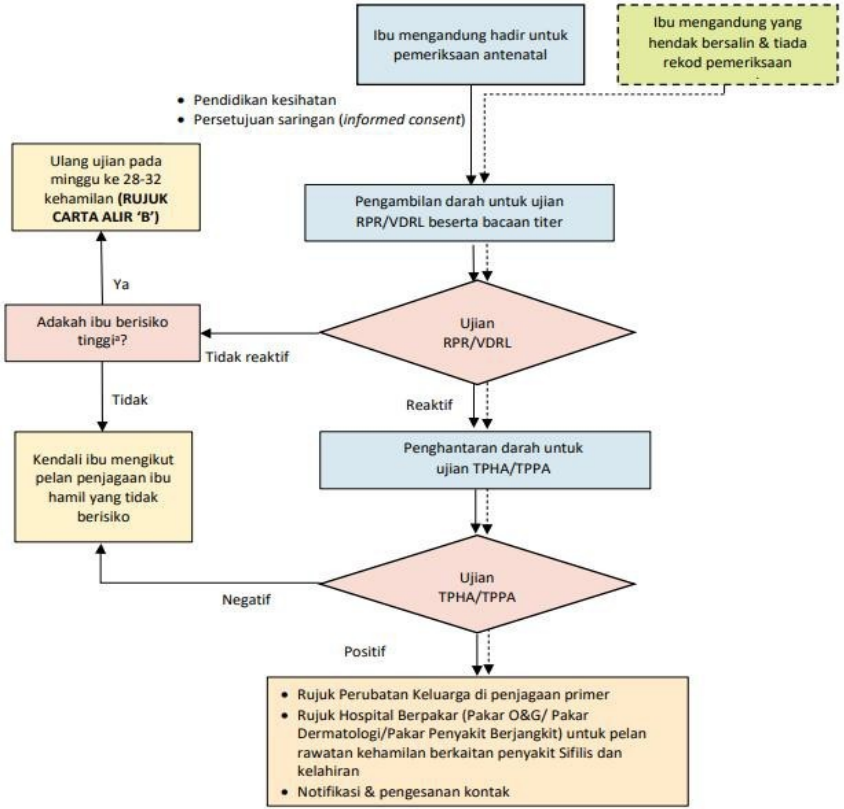
LABORATORY INTERPRETATION

Treponemal Antibody Test	RPR	TPPA	Interpretation
Non-Reactive	N/A	N/A	In the absence of high risk behaviour/exposure, syphilis is unlikely. Please correlate with clinical findings. Suggest to repeat testing after 2-4 weeks if clinically indicated.
Reactive	Reactive	N/A	Consistent with syphilis (past or current).
Reactive	Non-Reactive	Positive	Suggestive of syphilis infection (previously treated or untreated syphilis).
Reactive	Non-Reactive	Negative	Syphilis unlikely. If at risk for syphilis, repeat test in 2-4 weeks.

N/A = Not applicable

Laboratory investigations of syphilis in antenatal

Rajah 5A: Carta Alir Ujian Saringan Sifilis Ibu Mengandung Semasa Antenatal dan Intrapartum (Strategi Saringan Standard Berasaskan Makmal)



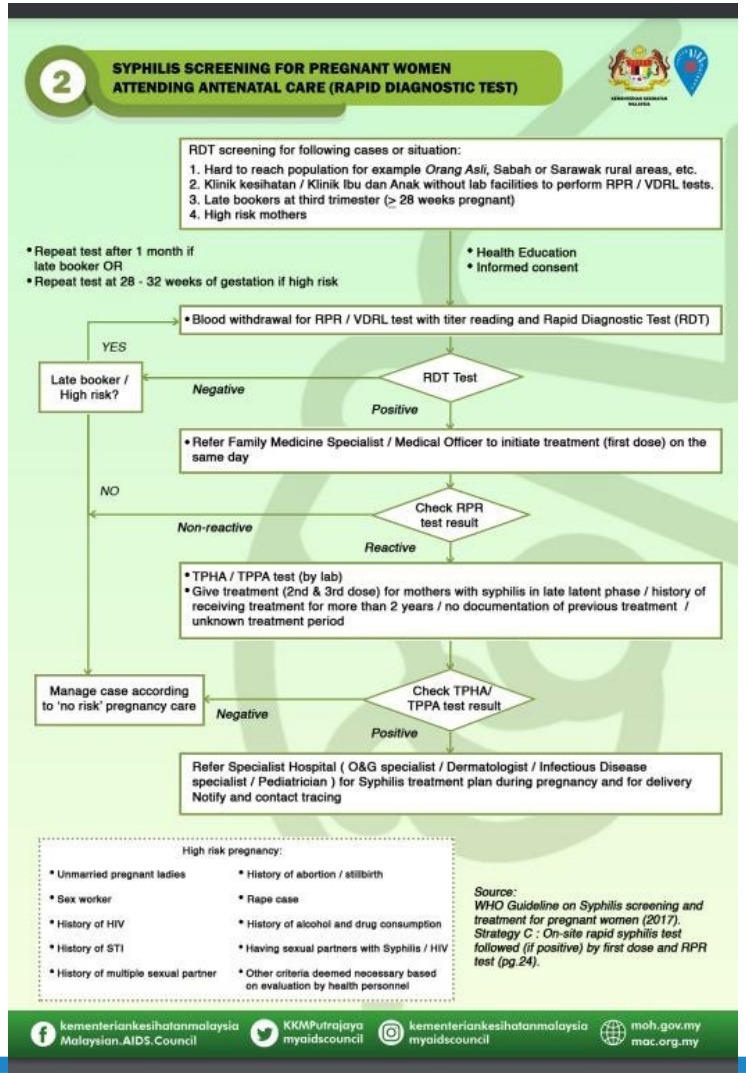
-Garis Panduan Pengukuhan Program Pencegahan Jangkitan HIV & Sifilis dari Ibu ke Anak Edisi Kedua 2021-

Role of RDT in antenatal screening

SURAT PEKELILING KETUA PENGARAH KESIHATAN MALAYSIA BIL. 10 /2021: PELAKSANAAN PENGUKUHAN PROGRAM PENCEGAHAN JANGKITAN HIV DAN SIFILIS DARI IBU-KE-ANAK

4. PENAMBAHBAIKAN TERHADAP GARIS PANDUAN

4.1 Ujian saringan HIV dan Sifilis hendaklah dijadikan sebagai salah satu ujian rutin dalam jagaan antenatal di semua fasiliti kesihatan kerajaan dan juga swasta. Penggunaan kit ujian pantas (*Rapid Diagnostic Test*-RDT) adalah digalakkan agar ujian pengesahan dan rawatan dapat dilakukan pada hari yang sama.


2
SYPHILIS SCREENING FOR PREGNANT WOMEN ATTENDING ANTENATAL CARE (RAPID DIAGNOSTIC TEST)
RDT screening for following cases or situation:
1. Hard to reach population for example Orang Asli, Sabah or Sarawak rural areas, etc.
2. Klinik kesihatan / Klinik Ibu dan Anak without lab facilities to perform RPR / VDRL tests.
3. Late bookers at third trimester (> 28 weeks pregnant)
4. High risk mothers
• Repeat test after 1 month if late booker OR
• Repeat test at 28 - 32 weeks of gestation if high risk
• Health Education
• Informed consent
• Blood withdrawal for RPR / VDRL test with titer reading and Rapid Diagnostic Test (RDT)
YES
Late booker / High risk?
Negative
RDT Test
Positive
• Refer Family Medicine Specialist / Medical Officer to initiate treatment (first dose) on the same day
NO
Non-reactive
Check RPR test result
Reactive
• TPHA / TPPA test (by lab)
• Give treatment (2nd & 3rd dose) for mothers with syphilis in late latent phase / history of receiving treatment for more than 2 years / no documentation of previous treatment / unknown treatment period
Manage case according to 'no risk' pregnancy care
Negative
Check TPHA/ TPPA test result
Positive
Refer Specialist Hospital (O&G specialist / Dermatologist / Infectious Disease specialist / Pediatrician) for Syphilis treatment plan during pregnancy and for delivery
Notify and contact tracing
High risk pregnancy:
• Unmarried pregnant ladies
• Sex worker
• History of HIV
• History of STI
• History of multiple sexual partner
• History of abortion / stillbirth
• Rape case
• History of alcohol and drug consumption
• Having sexual partners with Syphilis / HIV
• Other criteria deemed necessary based on evaluation by health personnel
Source:
WHO Guideline on Syphilis screening and treatment for pregnant women (2017).
Strategy C : On-site rapid syphilis test followed (if positive) by first dose and RPR test (pg.24).
kementeriankesihatanmalaysia
Malaysian.AIDS.Council
KKMPutrajaya
myaidscouncil
kementeriankesihatanmalaysia
myaidscouncil
moh.gov.my
mac.org.my

Laboratory diagnosis of Congenital Syphilis

Who Global surveillance case definition for congenital syphilis

Microbiological evidence of congenital syphilis infection includes any one of the following:

-Demonstration by dark field microscopy or fluorescent antibody detection of T. pallidum in the umbilical cord, the placenta, a nasal discharge or skin material;

-Detection of T. pallidum-specific IgM;

-Infant with a positive non-treponemal serology titer ≥ 4-fold above that of the mother

Global guidance on criteria and processes for validation: elimination of mother-to-child transmission (EMTCT) of HIV and syphilis. Geneva: World Health Organization; 2014

Diagnosis of congenital syphilis

1) Direct demonstration of spirochetes in body fluids or tissue
2) An infant's serum quantitative nontreponemal serologic titer that is fourfold higher than the mother's titer (RPR)*

*

- Screening on infant cord blood is not reliable
- Both mother and infant sampling should be at the same time and tested in same laboratory

Diagnosis of Congenital Syphilis

- Can be difficult because maternal immunoglobulin G (IgG) antibodies can be transferred through the placenta to the fetus

3.3 Bagi memastikan Malaysia terus mengekalkan status eliminasi MTCT Sifilis, Jawatankuasa Penyelaras Kebangsaan bagi eliminasi jangkitan HIV dan Sifilis dari ibu-ke-anak bil. 01/2021 pada 18 Mac 2021 telah mengenalpasti beberapa kelemahan yang perlu ditangani segera iaitu:

3.3.1 Kelewatan ujian pengesahan Sifilis (TPPA/TPHA).

Didapati banyak fasiliti kesihatan tidak mengambil sampel untuk ujian pengesahan Sifilis (TPPA/TPHA) pada hari yang sama semasa ujian saringan Sifilis (RPR) didapati reaktif. Ini telah menyebabkan ibu mengandung yang lewat membuat *antenatal booking* terlepas peluang untuk menerima rawatan lengkap (sekurang-kurangnya satu dos IM Bezathine Penicillin diberikan kepada ibu mengandung 30 hari atau lebih sebelum kelahiran) bagi mencegah jangkitan Sifilis kepada bayi.

3.3.2 Pemantauan rawatan semasa mengandung dan semasa lahir.

Pemantauan ujian serologi RPR ke atas ibu yang menerima rawatan serta sejurus selepas kelahiran tidak dilakukan. Ini menyebabkan kegagalan untuk mengesan kemungkinan berlakunya jangkitan semula Sifilis dan rawatan ulangan tidak diberikan semasa mengandung. Disamping itu, kegagalan memperolehi titer ujian RPR ibu sejurus selepas kelahiran telah menyukarkan perbandingan titer ibu dan bayi yang perlu dibuat. Perbandingan titer RPR bayi terhadap titer RPR ibu menjadi petunjuk kepada keberkesanan rawatan untuk mencegah *Congenital Syphilis*.

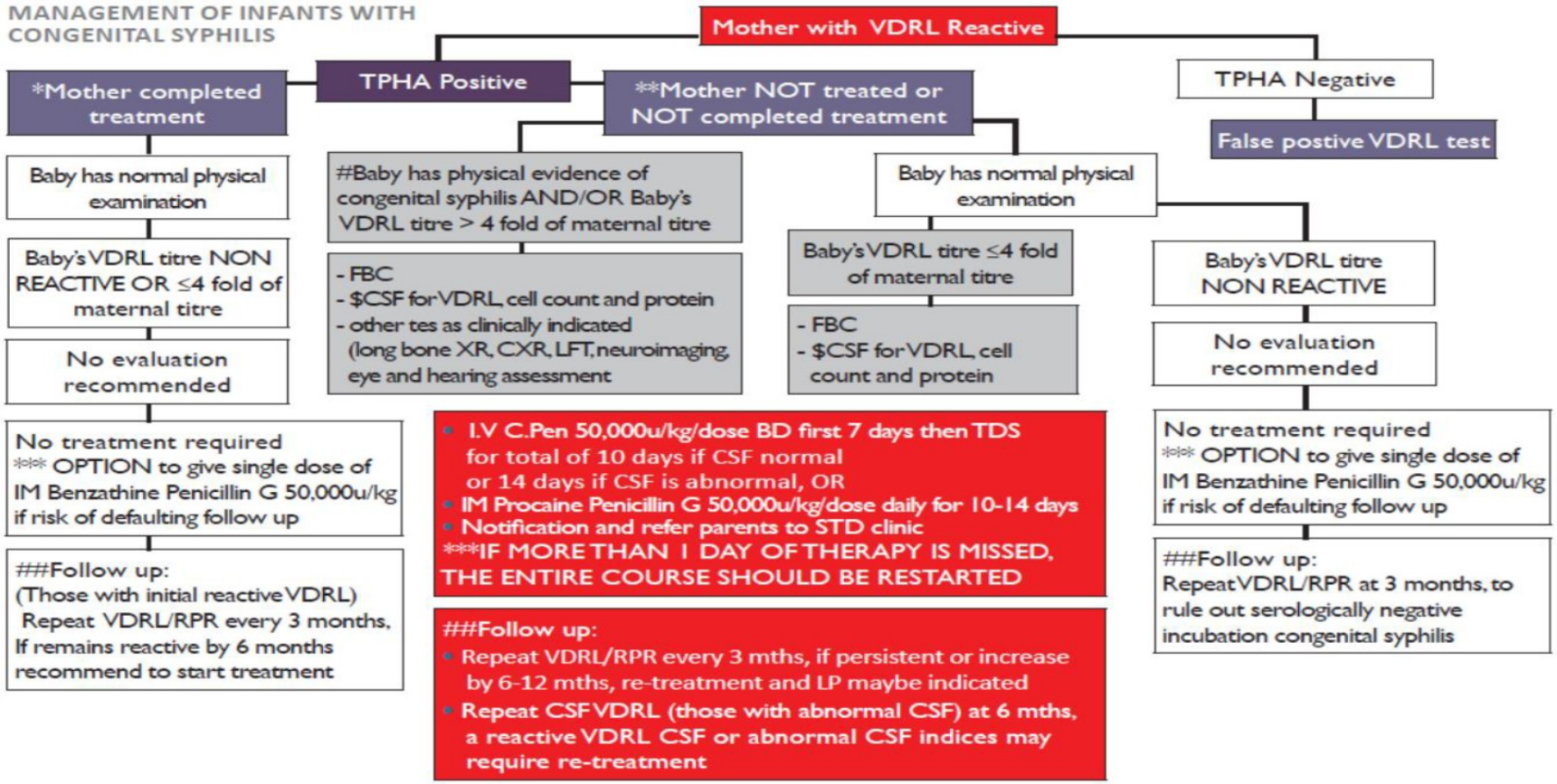
Laboratory diagnosis of Congenital Syphilis

Take mother's RPR titers at delivery and neonatal RPR titers preferably conducted by the same laboratory.

Do not take blood from umbilical cord blood

- contaminated with maternal blood and yield a false-positive result
- Wharton's jelly within the umbilical cord can yield a false-negative result.

Rajah 7: Carta alir pengurusan kes dan rawatan bayi yang dilahirkan oleh ibu Sifilis positif



Excerpt from Paediatric Protocols for Malaysian Hospitals, 4th Edition, Ministry of Health, 2018. Section 2: Neonatology. Chapter 30: Congenital Syphilis, pg 169.

Assuring quality in laboratory testing

- **Quality control on test kits**
  - Internal quality control
  - External quality control
- **Proficiency of users**
  - Training
  - Clear SOP


Thank You