METHOTREXATE

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History

METHOTREXATE

MTX was created by Yellapragada Subbarow (12 January 1895 – 8 August 1948) an Indian biochemist who also discovered the function of adenosine triphosphate (ATP) and Folic acid. Subbarow died in the United States due to cardiac arrest.

Folic Acids are essential for the synthesis of DNA (esp nucleoside purine and pyrimidine), RNA, thymidylates, and proteins.

Research has found that Folic Acid worsened outcome of leukemic patient, in contrast restriction led to improved prognosis. This has led to discovery of folic acid analog in 1947, "Methotrexate" (previously known as amethopterin) which acts as antifolate agent.

MTX was initially developed for anticancer drugs. Then it was widely used for treating autoimmune diseases, ectopic pregnancy, and for medical abortions.

MTX testing in SJMC

SJMC is renowned as a cancer treatment provider in the region and Laboratory must offer high quality services to the patients.

Throughout the years, MTX testing has evolved in SJMC

TDx FLx

- Fluorescence Polarization Immunoassay (FPIA)

Abbott TDx

Year: 1981
Company: Abbott Laboratories
Category: Immunoassay

Abbott ARCHITECT-

mmunoassay CMIA)

e Immunoassay

ARCHITECT
Flexible Solutions for Your Evolving Laboratory

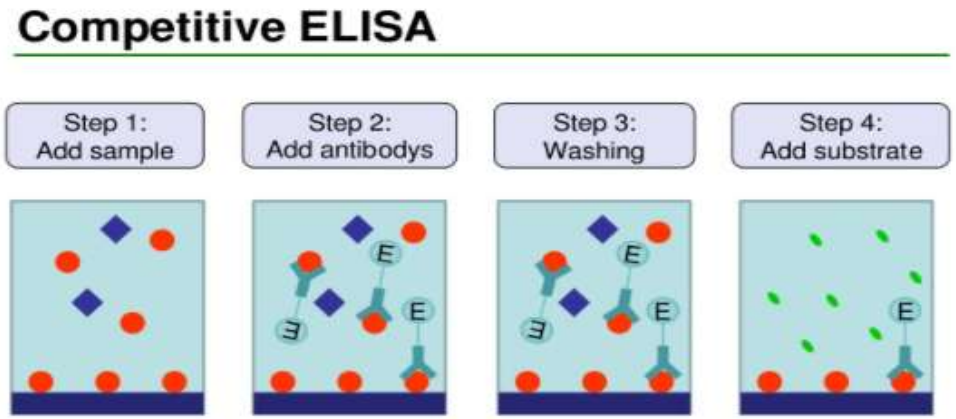
2020

GlobalHealth Asia-Pacific - Subang Jaya Medical Centre wins Oncology (Surgical) Service Provider of the Year in Asia Pacific

ARK-Methotrexate assay on c501

Developed by third party manufacturer, ARK Diagnostic

Homogeneous Competitive Enzyme Immunoassay



- Patients sample/ MTX
- MTX labelled G6PD and enzyme in R2
- Rabbit polyclonal antibody to MTX- in R1
- Substrate NAD-in R1

Limitation

- Reagent preparation
- QC and Calibration Stability influenced by instrument maintenance
- Low detection limit (0.04-1.2 umol/L)
- Expensive (Reagent (only 70 tests), QC each 2 mL, Calibrators, Diluent 25 mL)
- Requires Manual Dilution (where necessary)
- 6-point Calibration consumes 12 tests – low volume reagent is not suitable for calibration.
- Reagent can cross react with DAMPA, result from metabolism of MTX by glucarpidase.
- Serum and plasma samples should not be interchanged. For consistency, using the same specimen matrix for individual patients.

Importance of MTX testing

Monitoring of plasma MTX level is very important to improve the safety of high dose (HDMTX) therapy. MTX levels should be followed until the plasma level is less than 0.1 µmol/L.

Patient receiving HDMTX might risk MTX toxicity especially to their kidneys (nephrotoxic).

Therefore MTX test must be run in urgent manner in order to facilitate “rescue therapy” when a high result is reported to doctors.

Rescue therapy inclusive of administration of Leucovorin/ Folinic acid (folic acid analog) which displaces MTX from binding to serum proteins and protects other normal cells.

Other therapy methods include adequate hydration promote diuresis and to prevent intratubular precipitation of MTX, urine alkalanization.

Why is MTX testing IMPORTANT?

MONITORING

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TOXICITY-NEPHROTOXIC

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URGENTLY RUN

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HDMTX –testing issue

- MTX testing on c501 has small detection range and thus sample with flagging: Samp? (high result exceeding measuring range) must be followed with manual dilution of factor 10 and so forth.
- Note that MTX assay on c501 has measuring range of between 0.04-1.2 umol/L.
- Patient with high dose of MTX may have MTX concentration up to 150 umol/L (therefore you have to perform 10 fold dilution, not 2 fold dilution!)
- In HMIS, Dr/nurse will mention 20hr in Request Note- indicating patient has been receiving high dose MTX as HDMTX . Testing at 20hr is critical to assure the renal clearance of drugs and execution of rescue therapy.

What to do next when you have Samp? error

According to reagent inserts, serial dilution should be performed.

Manual Dilution Protocol

The measurement range of the ARK Methotrexate Assay is 0.04 - 1.20 µmol/L. Specimens and controls containing methotrexate in higher concentrations (>1.20 µmol/L), are assayed by dilution of the specimen and controls into the measurement range.

Manually dilute the high specimen or control with ARK Methotrexate Dilution Buffer by preparing the appropriate ten-fold serial dilution as shown below.

	Sample Volume	Dilution Buffer Volume	Dilution	Dilution Factor
50 µL	Undiluted sample	450 µL	1:10	10
50 µL	1:10 sample	450 µL	1:100	100
50 µL	1:100 sample	450 µL	1:1000	1000
50 µL	1:1000 sample	450 µL	1:10000	10000

$$\text{Manual Dilution Factor} = \frac{(\text{Volume of Specimen} + \text{Volume of Dilution Buffer})}{\text{Specimen Volume}}$$

Multiply the assayed result by the dilution factor.

Alternative rescue therapy

MTX overdoses may lead to life-threatening complications in which Leucovorin rescue alone may not be sufficient. In these situations, where inordinately high MTX concentrations (>=10.5 umol/L) persist, a recombinant derivative of the bacterial enzyme carboxypeptidase-G2 has been used successfully in experimental clinical settings to prevent life-threatening complications. The enzyme works by rapidly hydrolyzing MTX into the inactive metabolites 4-deoxy4-amino-N’10 methylpteroic acid and glutamate .

However, specimens from patients who have received glucarpidase (carboxypeptidase G2) should not be tested with the ARK Methotrexate Assay.

Discussion

1. You have received sample from patient HDMTX at 20hr. Neat run result is flagged “Samp?”.

a. What should you do next?

Answer: proceed with 1.:10 dilution

b. You have followed dilution protocols as recommended. Samples with dilution factor 10 and 100 produce Samp? error. Then you proceed with 1000 dilution factor. But sample with dilution factor 1000 gives result 200 <test (flagged/ too diluted). What should you do next?

Answer: try to dilute 1:500 (i.e. between 100 to 1000 dilution factor)

Additional Information of Flagging on cobas 6000

No	Data Alarm	Alarm Definition
1	ABS?	Sample concentration is too high OR sample is lipaemic
2	>TEST	Sample concentration is too high
3	<TEST	Sample concentration is too low
4	>INDEX	HIL (hemolysis/ icteric/lipaemic) index warning
5	>PROZ	Prozone error
6	>LIN	Photometer lamp is deteriorating
7	>REACT	Sample concentration is too high
8	SAM?	Sample concentration is exceeding measuring range

Discussion (continue)

c. MLT Bob has accidentally spilled the diluent. The diluent volume available now is only 600 uL .he has only 100uL of patient plasma. How does he prepare 1:10 dilution without using up all his patient sample and by using limited volume of buffer ?

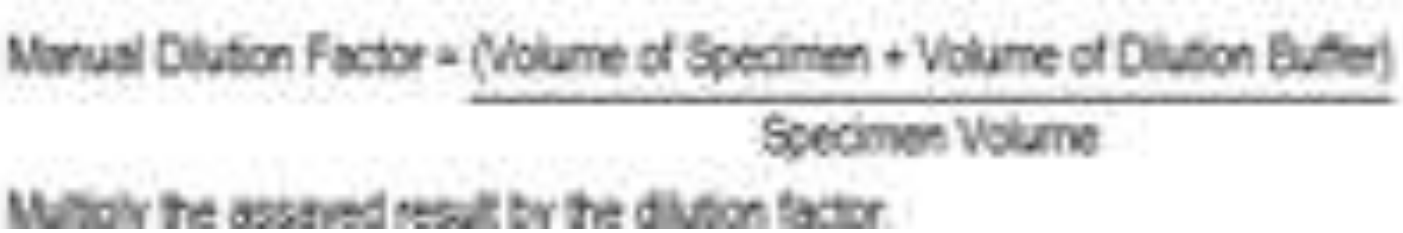
Answer: Bob can use 50uL of patient plasma

Therefore amount of buffer needed (Y) is to be calculated by following formula:

50 uL + Y = 50 uL x 10

Y = 500-50 uL

y = 450 uL



Conclusion

MTX testing plays important role in patient's health and safety . MTX results should be reported to requesting doctors in timely manner.

MLT should know what Samp? error meant and familiarized with dilution protocols in handling HDMTX at 20 hr. Failure of to do so will result in wastage of reagent.

Appreciate the role of MTX and Leucovorin.

Thank you for your attention!!
Good Luck in your Post CME test!!