COVID 19 Ag MICROLISA

Microwell ELISA Test for the Qualitative Detection of Covid-19 (SARS-COV-2) nucleocapsid antigen in Human nasopharyngeal, oropharyngeal, nasal swab in VTM and sample diluent

1. INTRODUCTION

Novel corona virus infection SARS CoV-2 [COVID-19] has spread to more than 203 countries of various regions including Africa, America, Europe, South East Asia and Western Pacific. The WHO had declared COVID-19 as the global public health emergency and subsequently as pandemic because of its worldwide spread. It is now one of the top-priority pathogens to be dealt with, because of high transmissibility, severe illness and associated mortality, wide geographical spread, lack of control measures with knowledge gaps in veterinary and human epidemiology, immunity and pathogenesis. The quick detection of cases and isolating them has become critical to contain it. Whereas molecular diagnostic tests were rapidly developed antigen based tests are of utmost requirement for early diagnosis of infection. Antigen is generally detectable in upper respiratory specimen during accute phase of infection.

2. INTENDED USE

Covid 19 Ag Microlisa is designed for in vitro qualitative detection of Covid 19 nucleocapcid antigen in human nasopharyngeal, nasal, oropharyngeal swab specimen collected in VTM or in Sample diluent from individuals who are suspected of COVID-19 . **The test is a screening test only and is an aid for the early diagnosis of SARS-CoV-2 infection in patient with clinical symptoms.** More specific and alternate diagnostic method should also be performed for confirmation of SARS -CoV-2 infection.

Caution: The laboratory results alone should not form the basis of medical report for individual patient. The clinical history and any other test performed must be taken into account. The presumptive diagnosis by Covid 19 Ag Microlisa may be confirmed by PCR.

3. PRINCIPLE

Covid 19 Ag Microlisa test is an enzyme immunoassay based on "Sandwich ELISA".

Anti-Covid 19 antibodies specific to nucleocapsid antigen are coated onto microtiter wells. Specimens and controls are added to the microtiter wells and incubated.

Covid antigen if present in the specimen, will bind to the Anti-Covid-19 antibodies absorbed onto the surface of the wells. The plate is then washed to remove unbound material. Horseradish peroxidase (HRP) conjugated with Anti-Covid-19 antibodies is added to each well. This conjugate will bind to covid antibody-antigen complex present in the microwells. Finally substrate solution containing chromogen and hydrogen peroxide is added to the wells and incubated. A blue colour will develop in proportion to the amount of Covid antigen present in the specimen. The colour reaction is stopped by a stop solution. The enzyme substrate reaction is read by EIA reader for absorbance at a wavelength of 450 nm and 630 nm. If the sample does not contain Covid antigen then enzyme conjugate will not bind and the solution in the wells will be either colourless or only a faint background colour develops.

4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2016.


Manufactured By


No. of tests

Lot Number
Batch Number


Manufacturing
Date


Expiry Date


Do not use if package
is damaged


Keep Dry

In vitro diagnostic
medical device


See Instruction
for use

Temperature
Limitation


Caution
See instruction for use

Catalogue Number


Keep away from sunlight

5. KIT PRESENTATION

- 96 Test Pack

6. KIT & ITS COMPONENTS

Microwells	Microwells coated with Anti-Covid 19 antibodies packed in a pouch provided with desiccant.
Lysis Buffer	Buffer containing surfactant and preservative for treatment of patient samples.
Sample Diluent	Buffer containing protein stabilizers and antimicrobial agents as preservative.
Enzyme Conjugate	Anti- Covid-19 antibodies labelled with horseradish peroxidase with protein stabilizers. Ready to use.
Wash Buffer Concentrate (25X)	PBS with surfactant. Dilute 1:25 with distilled water before use.
TMB Substrate	TMB solution. To be dilueted with TMB Diluent.
TMB Diluent	Buffer solution containing H_2O_2 with preservative
Control —	Ready to use, bovine serum albumin negative for Covid Antigen.
Control +	Ready to use, recombinant Covid nucleocapsid antigen with stabilizers and preservative.
Stop Solution	Ready to use, 1N sulfuric acid
Plate Sealers	Adhesive sheets to cover the microwells during incubation.
Microwell Frame	Plastic frame for Microwells
Clamp & Rod	For sealing microwell pouch after use
Sample Collection Tube	Vial for collection and treatment of patient samples.
Nasal Swabs	For collecting nasal & nasopharyngeal specimen

7. STORAGE AND STABILITY

Store the kit & its components at 2-8°C Expiry date on the kit indicates the date beyond which kit should not be used.

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- Micropipettes and microtips
- Elisa reader
- Distilled or deionized water
- Graduated Cylinders, for reagent dilution
- Paper towels or absorbent tissue
- Timer
- Elisa washer
- Incubator 37°C
- Disinfectant solution
- Disposable gloves

9. SPECIMEN COLLECTION & HANDLING

Human nasopharyngeal, oropheryngeal and nasal swab samples should be used for the test.

1. **Nasal swab**: Take the nasal swab provided in the kit and carefully insert into the nostril presenting the most secretion on visual inspection. Push the swab gently until the resistance is met into the nostril. Gently rotate the swab several times against the nasal wall and remove it from the nostril.
2. **Nasopharyngeal Swab sample collection**: Take the nasal swab provided in the kit and carefully insert into the nostril presenting the most secretion on visual inspection. Keep the swab near the septum floor of the nose by gently pushing the swab into the posterior nasopharynx. Gently rotate the swab several times against the nasal wall and remove it from the nasopharynx.
3. **Oropharyngeal Swab sample collection**: Take the oropharyngeal swab and depress the tongue so that the back of the throat can be seen. Rub the swab up and down against the back of the throat, avoiding the tongue and cheeks and gently remove it from the throat.
4. **Sample Transportation & Storage**: It is recomended to test the patient sample immediately after collection.

(i) Nasal / oropharyngeal /Nasopharyngeal Swab samples collected in VTM tube can be stored upto 72 hours at 2-8°C.**The swab samples should be collected prefrebally in 1 ml medium or less so as to reduce the dilution of the antigen present in patient specimen .**

(ii) Nasal / oropharyngeal/ Nasopharyngeal Swab samples collected in Sample Diluent provided in the kit should be run within 8 hours of collection.

(iii) If the sample is to be transported in viral transpor t medium (VTM), the VTM tubes shall be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

10. WARNING FOR USERS

CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of Disposable Gloves, glasses and proper portecting clothing is STRONGLY RECOMMENDED while running the test.
2. This test detects the presence of covid 19 antigen in the human nasopharyngeal, oropharyngeal and nasal swab specimens only. **Do not use Human Whole blood (EDTA Anticoagulated), plasma sera, urine and saliva as specimen for this test.**

3. Deviation in the test procedure may adversely affect test performance and/or produce invalid results and hence adherence to test procedure and precautions given in the product insert shall be strictly followed.
4. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
5. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
6. Do not pipette by mouth.
7. Tests are for in vitro diagnostic use only and should be run by competent trained person only.
8. All materials used in the assay and samples should be handles and disposed off in accordance with all local, state and national biosafety guidelines for handling & disposal of potentially infective material for Covid 19.
9. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
10. Observe established precautions against microbiological hazards throughout sample collection and testing procedures.
11. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. In case of needle prick or other skin puncture or wounds, wash the hands with excess of water and soap.
12. ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

11. PRECAUTIONS FOR USE

Optimal assay performance requires strict adherence to the assay procedure described in the manual.

1. Do not use kit components beyond the expiration date, which is printed on the kit.
2. Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
3. Stop solution contains sulfuric acid. If sulfuric acid comes in contact with the skin, wash thoroughly with water. In case of contact with eyes, flush with excess of water.
4. Use separate tips for TMB substrate and TMB diluent.
5. Prepare working substrate solution just 10 minutes prior to adding in the wells.
6. If blue colour or white particles appears in working substrate solution then do not use it. Take fresh containers and tips and prepare it again.
7. Do not allow microwells to dry once the assay has started.
8. Ensure that the microwell strips are levelled in the strip holder. Before reading, wipe the bottom of the microwell strips carefully with soft, absorbent tissue to remove any moisture.
9. A microwell reader which contains a reference filter with settings at 620 or 630 nm should be used. Use of a reference filter minimises interference due to microwells that are opaque, scratched or irregular.
10. Distilled or deionised water must be used for wash buffer preparation.
11. Bring all the reagents to room temperature (20-30^{o}C) before use.
12. Do not combine reagents form different batches, as they are optimized for individual batch to give best results.
13. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
14. Run negative and positive controls in each assay.
15. Use a separate tip for each sample and control and then discard it as biohazardous waste.
16. Thorough washing of the wells is critical to the performance of the assay.
17. Avoid strong light exposure during the assay.

13. PREPARATION OF REAGENTS

Prepare the following reagents before or during assay procedures. Reagents and samples should be at room temperature (20-30^{o}C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8^{o}C after use. All containers used for preparation of reagents must be cleaned throughly and rinsed with distilled or deionized water. Prewarm the incubator to 37^{o}C.

i) Anti-Covid 19 Antibodies coated strip

Bring foil pack to room temperature (20-30^{o}C) before opening to prevent condensation on the microwell strips.

a. Break-off the required number of strips needed for the assay and place in the well holder. Take the strip holder with the required number of strips, taking into account that two negative & one positive control should be included in each run while opening the fresh kit as well as each subsequent run.

b. **Unused wells should be stored at 2-8^{o}C, with dessicant in a aluminium pouch with clamp & rod. Microwells are stable for 30 days at 2-8^{o}C from the date of opening of sealed pouch, when stored with desicant along with clamp & rod.**

Caution: Handle microwell strip with care. Do not touch the bottom exterior surface of the wells.

ii) Preparation of Working Wash Buffer:

a) Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37^{0}C until all crystals dissolve.

b) Prepare at least 25ml. (1ml. concentrated buffer with 24 ml. water) of buffer for each strip used. Mix well before use.

c) Mix 20 ml. of 25X wash buffer concentrate with 480 ml. of distilled or deionized water. Wash buffer is stable for 2 months when stored at 2-8°C.

iii) Preparation of working substrate solution :

Mix TMB substrate and TMB Diluent in 1:1 ratio to prepare working substrate.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate (ml)	1	2	3	4	5	6	7	8	9	10	11	12
TMB Diluent (ml)	1	2	3	4	5	6	7	8	9	10	11	12

Do not store working substrate. Prepare a fresh dilution for each assay in a clean plastic/glass vessel. Determine the quantity of working substrate solution to be prepared from table. Mix solution thoroughly before use. Discard unused solution. A deep blue color present in the substrate solution indicates that the solution has been contaminated and must be discarded.

14. REAGENT STABILITY

Reagent	Preparation	Stability of opened/ diluted reagents (+2^{o}C to +8^{o}C)
1. Covid-19 antibodies coated Microwells	Ready to use	30 days
2. Working Wash Buffer	Dilute 1: 25 (1+24) with distilled water	2 months.
3. TMB Substrate & TMB Diluent	Dilute 1:1 in TMB Diluent just before use	Discard unused solution. A deep blue colour present in the substrate solution indicates that the solution has been contaminated and must be discarded.

15. PROCEDURAL NOTES:

1. Material should not be used after the expiry date shown on the labels. Components and test specimen should be at room temperature (20-30^{o}C) before testing begins. Return the kit and reagents to 2-8^{o}C after use.
2. All reagents must be mixed well before use.
3. To avoid contamination, do not touch the top or bottom of strips or edge of wells.
4. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
5. Prevent evaporation during sample incubation by covering the strips with sealer; remove sealer before washing.
6. Routine maintenance of wash system is strongly recommended to prevent carry over from highly reactive specimens to non reactive specimens.

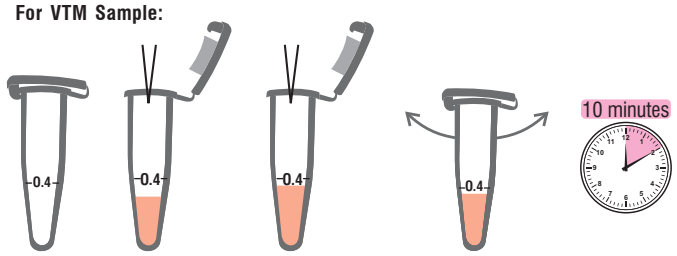
16. TEST PROCEDURE

Once the assay has started, complete the procedure without interruption. All the reagents should be dispensed in the centre of the well and the tip of the pipette should not touch the wall of the microwell.

Fit the stripholder with the required number of Anti-Covid 19 Antibodies coated strips. The sequence of the procedure must be carefully followed. Arrange the assay control wells in a horizontal or vertical configuration. Configuration is dependent upon reader software.

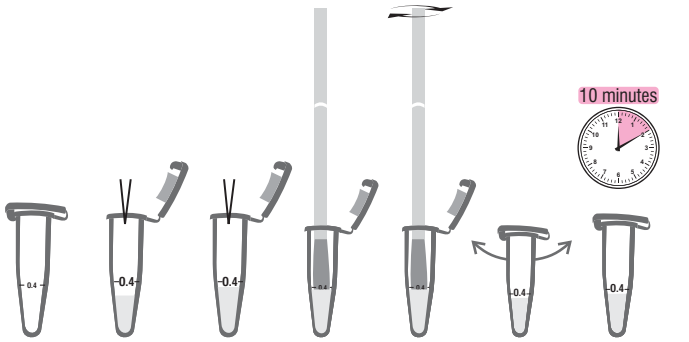
16.1 Sample Treatment:

i) For VTM Sample:



Take sample collection tube provided with the kit. | Add 300 µl VTM sample into the tube | Add 50 µl Lysis Buffer into the tube | Close the cap tightly and mix the tube on vortexer or by gentle shaking (5 times) | Incubate the tube containing sample and reagent for 10 minutes

ii) **For nasal swab, Nasopharyngeal & oropharyngeal swab sample:**



Take sample collection tube provided with the kit. | Add 300 µl Sample Diluent into the tube | Add 50 µl Lysis Buffer into the tube | Place patient swab sample into the reagent tube | Swirl the swab atleast 10 times while pressing the head against the bottom and side of the tube | Close the cap tightly and mix the tube on vortexer or by gentle shaking (5 times) | Discard the swab and incubate tube containing sample and reagent for 10 minutes

16.2 ELISA Procedure:

1. Add 200µl Negative Control in each well no. A-1 & B-1 respectively.
3. Add 200µl Positive Control in C-1 well.
4. Add 200 µl treated sample (from step 16.1) in each well starting from D-1.
5. Apply cover seal.
6. Incubate at 37°C ± 2°C for 60 min. ± 2 min.
7. While the samples are incubating, prepare working Wash Solution as specified in preparation of reagents.
8. Take out the plate from the incubator after the incubation time is over and, wash the wells 5 times with working Wash Solution.
9. Add 180 µl of Enzyme Conjugate Solution in each well.
10. Apply cover seal.
11. Incubate at 37°C ± 1°C for 30 min ± 2 min.
12. Aspirate and wash as described in step no.8.
13. Add 200 µl of working substrate solution in each well.
14. Incubate at room temperature (20-30°C) for 15 minutes in dark.
15. Add 100 µl of Stop Solution.
16. Read absorbance at 450 nm and 630 nm (reference filter) within 30 minutes in ELISA READER.

TEST VALIDITY:

Ensure the following is within specified acceptance criteria

i) NC or NCx O.D. must be < 0.15. If it is not so, the run is invalid and must be repeated.

ii) PC O.D. must be > 1.1. If it is not so, the run is invalid and must be repeated.

17. CALCULATION OF RESULTS

a. Cut off value = NCx + 0.15

b. Calculation of sample O.D. ratio : Calculate sample O.D. ratio as follows:

$$\text{Sample O.D. ratio} = \frac{\text{Sample O.D.}}{\text{Cut off Value}}$$

c. Calculation of Covid antigen units : Calculate by multiplying the sample O.D. ratio by 10.

Covid antigen units = sample O.D. ratio x 10.

e.g. : sample absorbance (O.D.) = 1.316

Cut off value = 0.220

Sample O.D. ratio = 1.316 / 0.220 = 5.981

Covid antigen units = 5.981 x 10 = 59.81

18. INTERPRETATION OF RESULTS

a. If Covid antigen unit is < 9 then interpret the sample as Negative for Covid antigen.

b. If Covid antigen unit is between 9 - 11 then interpret the sample as Equivocal for Covid antigen and should be retested in duplicate. If both duplicate retest sample antigen units are less than 9, then interpret as covid 19 Ag negative and if more than 11, then interpret as covid 19 Ag positive.

c. If Covid antigen unit is > 11 then interpret the sample as Positive for Covid antigen.

19. PERFORMANCE CHARACTERISTICS

In-House Evaluation

The performance of Covid 19 Ag Microlisa has been evaluated in-house withwith RT PCR Confirmed Clinical Patient Samples. The sensitivity was checked with 38 RT PCR Positive samples (up to CT 35) and Specificity was checked with 30 RT PCR negative samples. The results obtained are as follows:

S.No.	No. of Positive Sample & CT value	Covid 19 Ag Microlisa Result (Positive)	Covid 19 Ag Microlisa Result (Negative)
1.	17(14-20)	17	-
2.	15(20.5-25)	15	-
3.	6(26-35)	6	-

S.No.	No. of Negative Sample	Covid 19 Ag Microlisa Result (Positive)	Covid 19 Ag Microlisa Result (Negative)
1.	Negative samples (30)	-	30

Sensitivity : 100% **Specificity : 100%**

20. LIMITATION OF THE TEST

1. The test is for in vitro diagnostic use only.
2. The kit works best when used with fresh specimens. Specimens containing mucus/ particulates can give erratic results.
3. Potentially interfering substances like Nasal Sprays, nasal drops , Homeopathic allergic release medicines, anti inflammatory medicines ,anti- viral drugs ,Biotin etc. may lead to False positive results .
4. Any deviation from test procedure may lead to erratic results.
5. This is only a screening test and should not be used as the sole criteria for the diagnosis of Covid- 19 infection.
6. All samples detected non-reactive must be confirmed by using RT-PCR. Therefore for a definitive diagnosis & more accuracy of immune status, the patient's clinical history and symptomatology should be considered in order to obtain a confirmation of Covid-19 infection. A negative result at any time does not preclude the possibility of an early infection of Covid-19 infection. The results should be reported only after complying with above procedure.
7. This is a Qualitative test and therefore neither determine Covid 19 Antigen quantitative value nor the rate of SARS-CoV-2 antigen concentration.
8. For more accuracy of immune status, additional follow-up testing using other laboratory methods is recommended.
9. The test result must always be evaluated with other data available to the physician.
10. A negative result may occur if the concentration of antigen in a specimen is below the detection limit of the test or if the specimen was collected or transported improperly. Therefore a negative test result does not eliminate the possibility of SARS-CoV-2 infection, and should be confirmed by RT-PCR.

11 Positive test results do not rule out co-infections with other pathogens.

21. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

22. REFERENCES

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2. Sohrabi C., Alsafi Z., O'Neill N., Khan M., Kerwan A., Al-Jabir A. World Health Organization declares global emergency: a review of the 2019 novel coronavirus (COVID-19) Int J Surg. 2020 doi: 10.1016/j.ijsu.2020.02.034.
3. Lauer, S.A., et. al. The incubation period of Coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application, Ann Intern Med. 2020.

23. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. No colour developed at the end of assay	a) Any one reagent has been added in wrong sequence.	Follow the procedure meticulously & repeat assay.
	b) Inactivated conjugate, improper storage	Check storage of enzyme conjugate and it should be free of any contamination.
	c) Microplate inactivated, due to improper storage	Keep unused strips in aluminium poly pouch with the dessicant pouch inside and proerly closed with clamp & rod provided.
	d) Inactivated substrate, improper storage or preparation	Use freshly prepared substrate solution. Recheck procedure, repeat assay
	e) Omission of any step in test procedure	Follow the procedure meticulously & repeat assay.
	f) Incorrect incubator temperature timing or pipetting	Check procedure, incubator temperature & repeat assay
	g) Improper preparation of reagents, error of dilution, improper mixing of reagents.	Follow the procedure meticulously & repeat assay.
2. High O.D. value of Negative control	a) Plate not stopped after 15 minutes of additing stop solution	Follow the procedure meticulously & repeat assay.
	b) Same microtip used for Positive and negative controls	Change micropipette tips while addition of negative/ positive control
	c) Nonspecific attachment/ binding of other reagent	If plates get scratches/ aberrations during washing, non specific proteins may bind while addition of next step.
3. Too much colour in all wells of the plate (high background)	a) Contaminated substrate	Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container.
	b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc.	Check the container and quality of water used for dilution. Use of glass distilled water is preferred.
	c) Over incubation of substrate and delay in addition of stop solution.	Follow the procedure meticulously.
	d) Insufficient washing. i) Washing not consistent ii) Filling volume not sufficient. iii) Insufficient no. of wash cycles. iv) Contaminated wash device	Check wash device, fill the well close to the top. After washing, blot the microwells on absorbent tissue. Follow wash protocol meticulously
	e) Use of wash solution from other manufacturer.	Use only Covid 19 Ag Microlisa wash solution.
	f) Working substrate not protected from light	Incubate the plate in dark after addition of substrate.
4. Poor reproducibility	a) Washing problems.	Check all 8 ports/ manifold for uniform flow of wash buffer. If there are blockage, clean the ports.
	b) Uncalibrated pipettes or tips not well fitted, improper pipetting/ dispensing.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	c) Interference in optical pathway due to Air bubbles.	Clean or dry the bottom of microwells, check for bubbles and repeat the readings.
5. False Positive	Beside 3a, b, c, d, e & f incorrect interpretation and calculation of final results	Check the calculation part given in the insert and correctly interpret.
6. False Negative/ low O.D. of Positive control & positive sample	a) Inadequate addition of substrate/conjugate solution.	Follow the procedure meticulously & repeat assay.
	b) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.
	c) Microplate inactivated, due to improper storage	Keep unused strips in aluminium poly pouch with the dessicant pouch inside and proerly closed with clamp & rod provided.
	d) White particles in working substrate solution.	Discard the substrate and prepare the working substrate again in fresh tube.
	e) Uncalibrated pipettes, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	f) Deterioration of Enzyme conjugate	Check storage of Enzyme conjugate. It shall be stored at 2-8°C.
	g) Stop solution is added before 15 minutes. Reaction terminated before 15 minutes.	Follow the test procedure meticulously.
	h) O.D. taken at incorrect wavelength.	Read O.D. values at 450 nm and 630 nm.
	i) Inactivated sample/ improper sample Collection.	Follow the sample collection procedure meticulously and use sample within recomended time frame of collection.

in vitro diagnostic Reagent, not for medicinal use

VER-01 R-01

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