DECARBOXYLASE AND DIHYDROLASE TESTS ver. 0105

PRINCIPLE:

Decarboxylases attack specific amino acids and, by removing carboxyl groups, convert the amino acids to aliphatic amines forming CO2 as a second product. The decarboxylation of lysine and ornithine, resulting in the formation of cadaverine and putrescine respectively; and the production of ammonia by arginine dihydrolase, create an alkaline pH shift which changes the color of the indicator present in the tablet.

INGREDIENTS / MSDS:

The tablets contain the respective amino acids in a mixture of salts correctly buffered for each test and a pH indicator which changes color as the reaction progresses. The tablets do not contain any materials known at this time to be hazardous.

STORAGE:

Store tightly closed at room temperature.

MATERIALS REQUIRED:

All tests require fresh 24 hour growth on plated culture media. Consult the Manual of Clinical Microbiology for recommended media for the specimen. The following items are required but not provided: small glass tubes (12 X 75) for large tablets; inoculating loop; sterile pipette; & distilled water, pH 7.0-7.2.

Each substrate is sold separately.

K080 Arginine tablets - 50 ea
K1080 Arginine WEE-TABS - 28 ea
K380 Lysine tablets - 50 ea
K1380 Lysine WEE-TABS - 28 ea
K500 Ornithine tablets - 50 ea
K1500 ODC WEE-TABS
K475 Ninhydrin in DMSO - 4.0 ml.

SETUP PROCEDURE:

To perform the tests, a heavy suspension of the organism is made in distilled water in a small tube and the tablet is added. Use 0.5 ml water with the WEE-TAB and 1.0 ml water with the regular tablets. The tube is then incubated at 35-37oC. Use a glass tube if available. (See Discussion)

INTERPRETATION:

ARGININE-Arginine frequently reacts within 30 minutes with a positive red reaction from the phenol red indicator. Tests should be held 6 to 12 hours to verify negative. Ninhydrin is not used to confirm the arginine reaction.

LYSINE / ORNITHINE TABLETS Incubate the large tablets for 24 hours. WEE-TABS should be incubated for 4 hours. The brom cresol purple indicator in the lysine turns blue in a positive test. Ornithine has a phenol red indicator which starts out pink. If the test is negative, a yellow color will develop due to the acidification of the dextrose present in the medium. This will be overcome to produce a red color in a positive test. Positive reactions may be confirmed. (see Discussion)

DISCUSSION:

WEE-TAB results are final at 4-6 hours and confirmation is optional, although we recommend doing the ninhydrin procedure even then. WEE-TABS are packed in plastic tubes which chloroform will melt. If confirming, drop the small test tube into a larger glass one before adding the reagent. Large tablets are final at 24 hours. If the test is still yellow at 24 hours, the results are negative and no confirmation is required. If the lysine or ornithine test has changed at 24 hours, the Ninhydrin procedure MUST be done to confirm a true positive result. Some organisms (such as non-fermentors) produce an alkaline reaction from deamination of the amino acid causing the appearance of a positive decarboxylase reaction. The decarboxylation should be verified through the use of the ninhydrin reaction. While the amino acids are soluble in water, the amines are readily extracted into chloroform. If a solution of ninhydrin in chloroform is added to an aqueous solution of cadaverine or putrescine in a strongly alkaline medium, the amines move into the chloroform. This is indicated by the appearance of a deep violet color.

NINHYDRIN PROCEDURE:

1) Prepare the diluted Ninhydrin Reagent by adding 0.4 ml. of NINHYDRIN solution (K475) to 25 ml chloroform. The diluted reagent has the same expiration date as the original solution.

2) Add one drop of KOH followed by diluted Ninhydrin reagent in direct proportion to the test; 1 ml of reagent is used with the regular tablet, 0.5 ml with the strip test or WEE-TAB. Allow the tube to stand without shaking for 10-15 minutes. Development of a purple color in the lower chloroform layer is a positive test. Disregard the color which develops on the upper aqueous phase upon addition of the 40% KOH solution.

EXPECTED REACTIONS:

Organism	ARG	ODC	LDC
Enterobacter cloacae ATCC 13047	+	+	-
Klebsiella pneumoniae ATCC 33495	-	-	+
Serratia marcescens ATCC 8100	-	+	+

Consult the Manual of Clinical Microbiology for a more complete list of reactions for identifying organisms.

QUALITY CONTROL:

Each lot of tablets should be tested prior to use with organisms which produce known reactions such as those listed. The Manual of Clinical Microbiology should be consulted for detailed reactions and identification charts. All finished tests should be discarded in a manner conforming with accepted laboratory procedures for biohazardous material.

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