

PYRAZINAMIDASE (PYZ) K1537

DISCUSSION:

Elek and the guinea pig lethality tests have been used to determine whether a corynebacterium is pathogenic or non-pathogenic. (1) The guinea pig test is accurate but obviously undesirable. The Elek test is complicated, difficult to perform, and not always accurate.

In a clinical study of 99 cultures, using freshly prepared PYZ and cystine, " the three species of direct public health importance-namely *C. diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis* produced cystinase but did not produce pyrazinamidase. The other 38 cultures did not produce cystinase, and all but two, both cultures of *C. pseudodiphtheriticum*, produced pyrazinamidase." (2)

PRINCIPLE:

Most non-toxogenic species of corynebacterium possess the enzyme pyrazinamidase (pyrazine carboxylamidase) which catalyzes the hydrolysis of pyrazinamide into ammonia and pyrazinoic acid. The hydrolysis is detected by the addition of 20%(W/V) Ammonium ferrous sulphate hexahydrate solution in water. Toxogenic species are not able to hydrolyze the pyrazinamide.

MATERIAL SAFETY DATA:

Each tablet contains 2.5 mg. of pyrazinamide. This product does not contain any material known at this time to be hazardous. K980195 ferrous sulfate reagent is non-toxic when used as directed.

MATERIALS REQUIRED:

PYZ tablets are sold ready to use, one tablet per tube, 28 tubes per pack. All tests require fresh 24 hour growth. Consult the Manual of Clinical Microbiology for suggested media. Other required materials are a loop or swab for harvesting colonies, K980195 Ferrous sulfate (PYZ reagent), and purified water (pH. 6.5-7.5)

INSTRUCTIONS:

- 1) Add 5 drops of water to the tube containing the tablet.
- 2) Inoculate heavily using a visible paste of organism on the loop. Mix well.
- 3) Incubate at 35-37C for 4-24 hours.
- 4) Add 1-2 drops of prepared PYZ (ferrous sulfate) reagent.

REAGENT PREPARATION:

To prepare the reagent, remove the dropper assembly and fill to the top with water. Replace the assembly and shake to dissolve the tablet (or let stand about 30 minutes) before use. Prepared reagent should be good for approximately 30 days if kept refrigerated but should be rechecked for stability before each use. PYZ tests may be held up to one week for this purpose (see notation in INTERPRETATION)

INTERPRETATION:

An orange/red or brown color appearing instantly is a positive result indicating the organism is not a toxin producer. Negative results will be colorless or grey. Note: The positive reaction will fade upon sitting. In this case, if needed again, it can be restored (up to 3 times maximum) by adding another drop of reagent.

STORAGE:

Store tightly covered at 0-8 C.

QUALITY CONTROL:

PYZ tablets should be tested before use with organisms of known reactivity. We recommend *C. xerosis* ATCC 373 (positive) and *C. Diphtheriae* ATCC 13812 (negative). Discard used tests in a manner conforming with accepted laboratory procedures for biohazardous materials.

LIMITATIONS:

This test is an early screen only and should not be used as identification. All tests should be confirmed by other methods at a qualified laboratory.

REFERENCES AND FOOTNOTES:

- (1) Manual of Clinical Microbiology, 5th Edition, Chapter 31, page 280-283
- (2) Colman, Weaver, and Efstratiou, Screening Tests for Pathogenic Corynebacteria, Journal of Clinical Pathology, 1992: 45:46-48



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