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QUALITY CONTROL METHOD - 75

Preparation and Use of Oxoid BCET-RPLA Detection Procedure

Description:

This quality control procedure is designed to test for the presence of Bacillus cereus enterotoxin in strains that test positive for lecithinase and negative for Mannitol usage on QC-104 Mannitol Egg-Yolk Polymyxin agar or on the Oxoid Bacillus Cereus Detection Agar. This procedure should be performed by a trained laboratory technician.

Ingredients:

Oxoid BCET-RPLA

TD951 Sensitised Latex. Latex sensitised with specific *B. cereus* anti-enterotoxin (rabbit IgG).
TD952 Latex control. Latex suspension sensitised with non-immune rabbit globulins.
TD953 Enterotoxin control (lyophilized). Lyophilized *B. cereus* enterotoxin.
TD954 Diluent. Phosphate buffered saline containing bovine serum albumin.
Instruction leaflet

Brain Heart Infusion Broth (Oxoid, Criterion or BBL)
Sodium chloride solution (0.85% sterile)

Equipment and Supplies:

Sterile gloves
Lab coat
Enterotoxin positive strain of B. cereus or BT (AM uses Btk 679)
Enterotoxin negative strain of BT (AM uses Bti ATCC 700872)
Sterile Microtitre plates (V-well) and lids (recommend Thermo, Fisher, VWR or Weber)
Eppendorf or similar variable pipettor (10 to 100 µl) with sterile tips
25 µl diluter
Sterile screw-cap centrifuge tubes (Corning disposable used at AM, but Pyrex glass will work)
Refrigerator set at 4 deg. C
Centrifuge capable of generating 900g (typically 3000 rpm in a small bench top centrifuge)
for AM, use Vanguard model V-6500 at 3400 rpm)
Sodium hypochlorite solution (>1.3% w/w((disinfectant)
Environmental orbital shaking incubator (New Brunswick model G-24)
Micromixer with flat top attachment (optional)
Thermometer
1 cc sterile syringe

Laboratory balance sensitive to 0.001 g
Sterile individually packaged sterile inoculating loop-needles (blue), 1 µl, or 3 mm platinum loop, sterilized

Procedure:

1. Purchase ingredients from an approved source. .

2. Place a label on packages with expiration date marked in bold RED letters and store contents of BCET-RPLA kit in the refrigerator until used. Bring to room temperature before using and promptly seal and return remaining contents to refrigerator after use.

3. Inoculate the isolated organism into Brain Heart Infusion Broth (Hardy tubes with 5 ml in each) and incubate at 30-37°C for 6-18 hours with shaking set at 250 cycles/minute. If strains grown are halophilic (rare for Bacillus cereus), then Oxoid advised that addition of sterile saline to bring the media up to 0.5% salt should not interfere with results.

4. After growth, transfer to sterile screw-cap centrifuge tubes either centrifuge at 900g (Vanguard V-6500) for 20 minutes at 4˚C. **Retain the sediment for toxin assay.**

5. An optional alternative method of separation uses membrane filter using a 0.2µm-0.45µm low protein-binding filter is an optional procedure to centrifugation, but Alken-Murray staff found this harder to work with and maintain sterility. **Retain the filtrate for toxin assay and discard the supernatant**, if you used the filtration method.

6. Check the particular cultural method of use with a standard enterotoxin-producing strain such as *B. cereus* NCTC 11145. At Alken-Murray, we use *Bacillus thuringiensis kurstaki,* 679 as our enterotoxin positive control strain and use *Bacillus thuringiensis israelensis* 872 as our enterotoxin negative control strain.

7. The reconstituted toxin control will agglutinate the sensitised latex. The use of the toxin control will provide a reference for the positive patterns illustrated below (see interpretation of Test Results). The control should be used from time to time only to confirm the correct working of the test latex. The toxin control is not provided at a specified level and therefore must not be used as a means of quantifying the level of toxin detected in the test sample.

8. The latex reagents (TD951, TD952) and diluent (TD954) are ready for use. Shake the latex reagents thoroughly before use, to assure a homogenous suspension. Beware transferring bubbles formed when latex is shaken or test may not work properly.

9. Reconstitute the enterotoxin control by applying 0.5 ml of sterile diluent (TD954) to TD953. Shake gently until contents are dissolved. Reconstituted control is good for 3 months if kept refrigerated at 4 deg. C.

10. Arrange a microtitre plate so that each row consists of 8 wells. Each sample needs the

use of two rows, so use odd numbered rows for testing and even numbered rows for controls for each strain tested. Place plate on a folded (unused) black garbage bag on a stainless steel tray (so that it can be moved to a place where it will remain undisturbed for 24 hours after setup and mixing).

11. Using the Eppendorf (or similar) pipettor, dispense 25 µl of diluent (TD954) to wells 2 through 8 for two rows for each sample to be tested (each well in the pair of rows for each strain tested, EXCEPT the first well). For 3 samples and two control strains, you will need 10 rows. If the Enterotoxin positive control is also tested, you will need 12 rows.

12. Add 25 µl of each test sample to wells 1 and 2 for BOTH rows assigned to that sample

13. Starting with the SECOND well in each row (use a new tip for each row), pick up 25 µl and perform a doubling dilution through well number 7. Leave well 8 containing diluent ONLY. **Try to avoid forming bubbles during this procedure.**

14. Add 25 µl of sensitized latex (TD951) to ALL cells in odd numbered rows

15. Add 25 µl of latex control (TD952) to ALL cells in even numbered rows

16. Mix the contents in each well by gently agitating by hand or by using a micromixer set on a low setting with flat top attachment. Avoid spilling contents of wells.

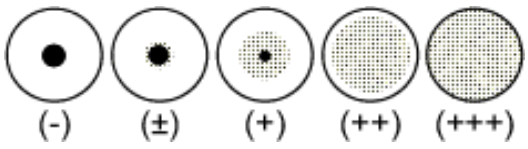
17. Cover the plate with a sterile lid and leave undisturbed for 24 hours.

18. Centrifuge tubes, membrane filters, microtitre plates, lids and pipette tips should be sterilised by autoclaving at 121°C for 15 minutes or disinfected, before disposal, in hypochlorite solutions(>1.3%w/w).
Dispose of culture extracts, food extracts, samples and enterotoxin controls in hypochlorite solution (>1.3%w/w).

Interpretation of Results:

1. Examine all wells in plate against black background after 24 hours.

2. The agglutination pattern should be judged by comparison with the following illustration:



3. Results classified as (+), (++) are considered to be positive. Results in the row of wells containing latex control (TD952) should be negative.

4. In some cases, non-specific agglutination may be observed. In such cases the results should be interpreted as positive, provided that the reaction with sensitised latex (TD951) is positive to a higher dilution of test sample that that seen with the latex control (TD952).

5. The last well in all rows should be negative. If positive patterns are observed in some of these wells, the reaction should be regarded as invalid.

Limitations of the Test:

1. The sensitivity of this test in detecting the enterotoxin is 2ng/ml in the test extract.

2. Production of enterotoxin in culture filtrate depends on the growth conditions. A positive result obtained in this way demonstrates the production of enterotoxin; it does not imply the *in vitro* production of toxins to those levels.

3. For AMH strains, Oxoid technical staff suggested that the addition of 0.1 ml of 0.85% saline to the BHI broth to encourage faster growth of halophilic strains would not interfere with the test

References:

1. Oxoid BCET-RPLA instruction manual and product insert.

2. Kramer, J.M. and Gilbert; R.J. (1988). *In Foodborne Bacterial Pathogens* (ed. M.P. Doyle) pp. 21-70 Marcel Dekker Inc,. New York

3. Hauge, S. (1955) *J. Appl. Bacterial* **18:** pp. 591-595.

4. Mortimer, P.R. and McGann, G. (1974). *Lancet* **1:** pp. 1043-1045

5. Turnbull, P.C.B. (1936). In Pharmacology of Bacterial Toxins (ed. F. Dorner and J. Drews) pp. 397-448. Pergamon Press, Oxford.

6. Holbrook, R. and Anderson, J.M. (1980). *Can. J. Microbiol.* **26:** pp. 753-759.