#### PROTOCOL

#### Setting up an API-20E strip test.

#### 1. Place a small amount of water in the incubation tray (under the API strip itself) to provide humidity during incubation.

#### 2. Prepare a milky (visibly turbid) suspension of your organism in sterile water.

#### 3. Use a plastic sterile pipette to half-fill test wells of your API strip with the above suspension (Holding the strip at a slight angle as well as letting the end of the pipette touch the side of a well will make this easier. Make sure all air bubbles are eliminated).

#### 4. When there is a line under a well (ADH, LDC, ODC, H2S, URE), add sterile mineral oil to completely fill in the well.

#### 5. When there is a box under a well (CIT, VP and GEL), add some more bacterial suspension to completely fill in the well.

#### 6. Place the plastic lid back on and label the tray with your name, date and organism code.

#### 7. Incubate at 37°C for 24 hours.

**After incubation:**

#### 1. Add one drop of reagent TDA (10% ferric chloride) to the well labelled as TDA.

#### 2. Add one drop of reagent IND (Kovacs’ reagent) to the well labelled as IND.

#### 3. Add one drop of reagent VP1 (40% KOH), THEN add one drop of reagent VP2 (6% alpha-naphtol) to the well labelled as VP.

#### 4. Wait for 10 min to observe colour changes.

#### 5. Read the colour reactions obtained for each well, and record your results (as positive or negative) for each test following the interpretation guide.

#### 6. Using the BacDive API test finder, try to determine the identity of your unknown based on your results.

#### TEST RESULTS – API-20E



#### A = even; B = odd

#### INTERPRETATION TABLE – API strips

**Table 1: scoring test and reaction results**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Scoring Test** | **Reaction tested** | **Negative** | **Doubtful** | **Positive** | **Strong Positive** |
| ONPG | β-galactosidase | colourless | faint yellow | pale yellow | Yellow |
| ADHLDCODC | Arginine dihydrolaseLysine decarboxylaseOrnithine decarboxylase | yellow | yellow/orange | orange/red | red   |
| CIT | Citrate utilisation | yellow | pale green | Blue | deep blue |
| H2S(deposit) | Sulphide production | no deposit | trace of black | Black | heavy black deposit |
| URE | Urease | yellow | yellow/orange | orange/red | Red |
| TDA (addreagent) | Tryptophan deaminase | yellow | yellow/brown | Brown | dark brown |
| IND (addreagent) | Indole production | yellow | pale pink ring | pink ring | red ring |
| VP (addreagent) | Voges-Proskauer reaction | colourless | pale pink | pink/red | Red |
| GEL | Gelatinase | pigment does not dissolve | pigment dissolves slightly | Grey | Black |
| GLUMANINOSORRHASACMELAMYARA | Glucose fermentationMannose fermentationInositol fermentationSorbose fermentationRhamnose fermentationSucrose fermentationMellibiose fermentationAmygdalin fermentationArabinose fermentation | blue | blue/green | Yellow | strong yellow |
| OXI | Oxidase | pale brown |  | dark brown | purple |