

Appendices

Appendix One: Additional Methodology

A1.1 Disc Diffusion

- Prepare Agar
- Prepare overnight nutrient broth

Preparing Agar.

Making agar fresh: Add 14g nutrient agar to 500ml bottle. Fill with distilled water leaving approx 3 inches of space at top. Place lid on bottle and tighten, shake to mix powder with water. Label bottle with autoclave tape. Loosen lid and place in autoclave. Repeat until desired number of bottles prepared. Switch on autoclave and leave for approx 2 hours. When complete check tape to ensure it has turned stripy and therefore indicates agar is sterilised. Add agar bottles to water bath at 55⁰C and leave for approx 45 minutes or until bottles have cooled enough to pour. Then, using aseptic technique pour the agar in to petri dishes, just enough to cover the bottom of the dish. Each bottle should fill approx 20 dishes. Spread dishes over counter surface to allow the agar to set more quickly. All agar plates should be set 30-45 minutes after pouring. Once the agar has set place the plates lid up in an incubator at 37⁰C overnight. Plates will then be ready to use the day after. Surplus plates can be kept refrigerated at 4⁰C.

Preparing overnight nutrient broth

In the meantime prepare the overnight nutrient broth. Take stock plate from fridge (4⁰C) Check gas is on and meter reads zero to determine it is safe to use gas. Switch on Bunsen;

make sure is on fire proof matt, switch to blue flame. Collect nutrient broth from rack in cupboard. Take loop and hold downwards into flame until it glows red, remove lid from broth (do not place lid on counter), flame and place loop inside, it should hiss as it cools. Flame jar briefly, using loop remove a single colony from the stock plate and place into jar, wiggle loop in jar, flame jar, place lid on and shake gently. Flame loop and place in safe area as to not burn anything. Write name, date, and microbe on jar, place in rack and into incubator at 37°C overnight. These should be remade before every experiment. Remember to flame before and after each transfer to avoid contamination of broth. To ensure nutrient broth is sterile to start with place an unopened jar from the batch in the rack with the experiment jars.

Back to Agar, if it is clear, remove from kettle after two hours, using gloves, it will be very hot and dangerous stand back from kettle to avoid hot steam. Tighten lid and invert gently approx 3 times, release pressure and place in water bath at 55°C for approx 45mins to cool. Once cooled remove from water bath, tighten lid, invert gently again and retighten lid. It will then be ready to pour into plates.

Pouring of plates

Collect 70% alcohol from cupboard, soak some tissue and wipe counter. Return alcohol to cupboard. Collect petri dishes from cardboard boxes and remove from plastic bag. Label the bottom of each petri dish. Turn on Bunsen burner, open agar jar, and flame lid very briefly to sterilise but not melt plastic rim of bottle. Place lid on counter facing up to avoid cross contamination. Pour agar into petri dishes working from the bottom of piles

of 5. Spread plates out on counter to set the agar for approx 30mins until the agar solidifies and turns opaque. Place petri dishes in incubator at 37°C overnight. If there is any agar left in the bottle, pour down sink with plenty of water and rinse bottle straight away. Place in box to be washed.

If propolis is to be tested it needs to be prepared the night before. Weigh propolis and dissolve in known volume of 80% ethanol. Weigh a piece of filter paper. Filter mixture and allow residue to dry, reweigh residue and the filter paper and calculate what percentage of propolis actually in solution. Prepare desired number of dilutions and dip required number of 13mm whatmann discs in each dilution. Place each batch of discs in labelled sterile petri dishes and place in refrigerator overnight at 4°C to allow ethanol to evaporate. Discs can then be used the next day.

Glucose Broth Recipe:

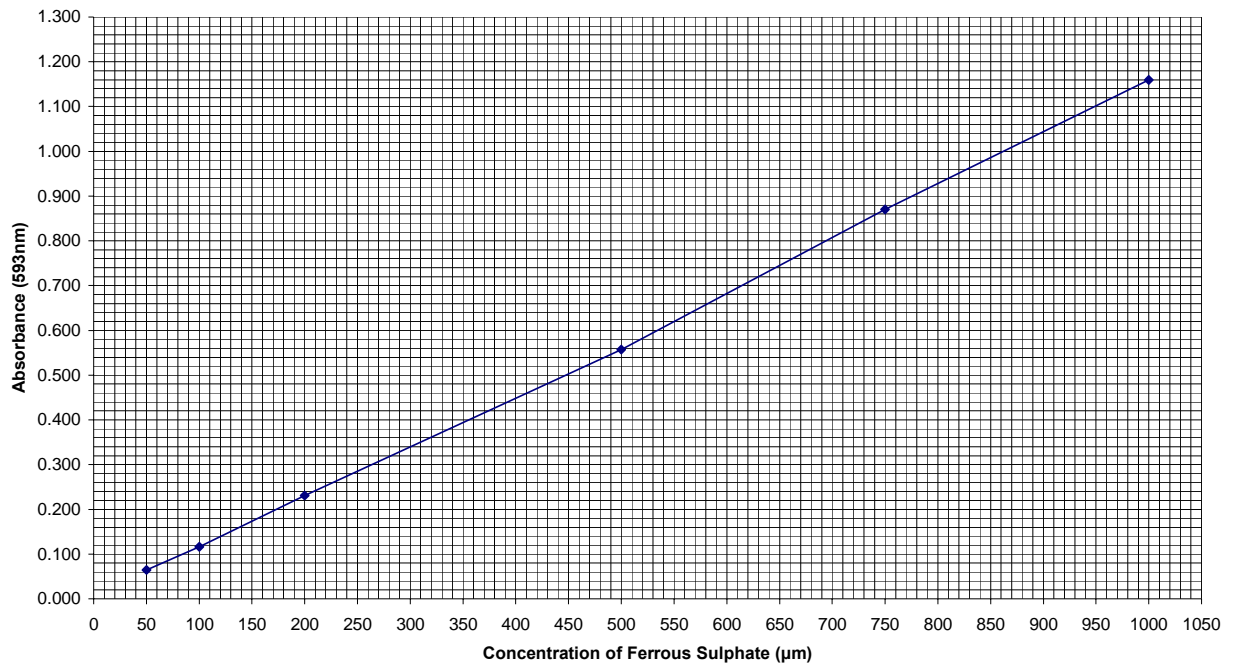
- 13.0g Oxoid CM1 broth powder
- 1.0g Glucose
- 3.68g K₂HPO₄
- 1.32g KH₂PO₄
- 1000ml Distilled H₂O

Adjust mixture to pH 7.2.

Source: <http://www.ncimb.com>

Appendix Two: Standard Curve

Curve showing Absorbance of Various Concentrations of Ferrous Sulphate Solution at 593nm



Appendix Three: Example of Raw Data (FRAP)

| Product | Dilution (%) | FRAP Value (μmol/l) | Product | Dilution (%) | FRAP Value (μmol/l) | Product | Dilution (%) | FRAP Value (μmol/l) | Product | Dilution (%) | FRAP Value (μmol/l) |
|----------------|---------------------|----------------------------|----------------|---------------------|----------------------------|------------------|---------------------|----------------------------|----------------|---------------------|----------------------------|
| Standard honey | 50 | 760 | Manuka 5+ | 50 | Error | Artificial Honey | 50 | 0 | Tea Tree 12+ | 50 | Error |
| Standard honey | 50 | 905 | Manuka 5+ | 50 | Error | Artificial Honey | 50 | 0 | Tea Tree 12+ | 50 | Error |
| Standard honey | 50 | 760 | Manuka 5+ | 50 | Error | Artificial Honey | 50 | 0 | Tea Tree 12+ | 50 | Error |
| Standard honey | 25 | 370 | Manuka 5+ | 25 | 650 | Artificial Honey | 25 | 0 | Tea Tree 12+ | 25 | 600 |
| Standard honey | 25 | 320 | Manuka 5+ | 25 | 630 | Artificial Honey | 25 | 0 | Tea Tree 12+ | 25 | 620 |
| Standard honey | 25 | 310 | Manuka 5+ | 25 | 640 | Artificial Honey | 25 | 0 | Tea Tree 12+ | 25 | 600 |
| Standard honey | 12.5 | 0 | Manuka 5+ | 12.5 | 350 | Artificial Honey | 12.5 | 0 | Tea Tree 12+ | 12.5 | 340 |
| Standard honey | 12.5 | 0 | Manuka 5+ | 12.5 | 360 | Artificial Honey | 12.5 | 0 | Tea Tree 12+ | 12.5 | 320 |
| Standard honey | 12.5 | 0 | Manuka 5+ | 12.5 | 350 | Artificial Honey | 12.5 | 0 | Tea Tree 12+ | 12.5 | 370 |
| Standard honey | 6.25 | 0 | Manuka 5+ | 6.25 | 160 | Artificial Honey | 6.25 | 0 | Tea Tree 12+ | 6.25 | 170 |
| Standard honey | 6.25 | 0 | Manuka 5+ | 6.25 | 165 | Artificial Honey | 6.25 | 0 | Tea Tree 12+ | 6.25 | 165 |
| Standard honey | 6.25 | 0 | Manuka 5+ | 6.25 | 170 | Artificial Honey | 6.25 | 0 | Tea Tree 12+ | 6.25 | 230 |
| Standard honey | 3.25 | 0 | Manuka 5+ | 3.25 | 100 | Artificial Honey | 3.25 | 0 | Tea Tree 12+ | 3.25 | 85 |
| Standard honey | 3.25 | 0 | Manuka 5+ | 3.25 | 85 | Artificial Honey | 3.25 | 0 | Tea Tree 12+ | 3.25 | 70 |
| Standard honey | 3.25 | 0 | Manuka 5+ | 3.25 | 85 | Artificial Honey | 3.25 | 0 | Tea Tree 12+ | 3.25 | 70 |

| Product | Dilution (%) | FRAP Value (µmol/l) | Product | Dilution (%) | FRAP Value (µmol/l) | Product | Dilution (%) | FRAP Value (µmol/l) |
|----------------|---------------------|----------------------------|----------------|---------------------|----------------------------|----------------|---------------------|----------------------------|
| Tea Tree 16+ | 50 | Error | Tea Tree 18+ | 50 | Error | Manuka 30+ | 50 | Error |
| Tea Tree 16+ | 50 | Error | Tea Tree 18+ | 50 | Error | Manuka 30+ | 50 | Error |
| Tea Tree 16+ | 50 | Error | Tea Tree 18+ | 50 | Error | Manuka 30+ | 50 | Error |
| Tea Tree 16+ | 25 | 605 | Tea Tree 18+ | 25 | 605 | Manuka 30+ | 25 | 670 |
| Tea Tree 16+ | 25 | 600 | Tea Tree 18+ | 25 | 600 | Manuka 30+ | 25 | 960 |
| Tea Tree 16+ | 25 | 620 | Tea Tree 18+ | 25 | 620 | Manuka 30+ | 25 | 700 |
| Tea Tree 16+ | 12.5 | 340 | Tea Tree 18+ | 12.5 | 340 | Manuka 30+ | 12.5 | 410 |
| Tea Tree 16+ | 12.5 | 350 | Tea Tree 18+ | 12.5 | 350 | Manuka 30+ | 12.5 | 650 |
| Tea Tree 16+ | 12.5 | 350 | Tea Tree 18+ | 12.5 | 350 | Manuka 30+ | 12.5 | 610 |
| Tea Tree 16+ | 6.25 | 180 | Tea Tree 18+ | 6.25 | 180 | Manuka 30+ | 6.25 | 180 |
| Tea Tree 16+ | 6.25 | 200 | Tea Tree 18+ | 6.25 | 200 | Manuka 30+ | 6.25 | 165 |
| Tea Tree 16+ | 6.25 | 180 | Tea Tree 18+ | 6.25 | 180 | Manuka 30+ | 6.25 | 240 |
| Tea Tree 16+ | 3.25 | 85 | Tea Tree 18+ | 3.25 | 85 | Manuka 30+ | 3.25 | 150 |
| Tea Tree 16+ | 3.25 | 70 | Tea Tree 18+ | 3.25 | 70 | Manuka 30+ | 3.25 | 165 |
| Tea Tree 16+ | 3.25 | 55 | Tea Tree 18+ | 3.25 | 55 | Manuka 30+ | 3.25 | 160 |

