|  |  |  |  |
| --- | --- | --- | --- |
| **SOP Number:** | M.7.50 | **Effective Date:** | 03/2013 |
| **Department:** | Microbiology | **Revision Date:** | 02/18/2013 |
| **Policy (P), Procedure (PR)or Both (P/P):** | PR | **Version:** | 2 |

|  |  |  |
| --- | --- | --- |
| Applicable Standards |  | Version History |
| Standard | Organization  |  | Version | Effective Date | Retired Date |
| MIC.11016 |  CAP |  | 1 | 10/2006 | 02/2013 |
| MIC.21626 |  CAP |  | 2 | 02/2013 |  |
|  |   |  |  |  |  |
|  |   |  |  |  |  |
| Related Documents |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |  |
| Review History (Up to the Last 15 Occurrences) |
| Date | Version | Revision Type | Review By/Initials & Date |
| 02/2013 | 2 |  Director Review  | J. Lewis 2/21/2013 |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |

|  |
| --- |
| Distribution |
| CSHCC Shoreline Microbiology SOP |
| CSHCC Memorial Microbiology SOP |
|  |
|  |
|  |
|  |
|  |

**PRINCIPLE:**

Remel RapID ANA II System is a qualitative micromethod employing conventional and chromogenic substrates for the identification of anaerobic bacteria isolated from human clinical specimens.

The RapId ANA II System is comprised of RapID ANA II Panels and RapID ANA II Reagent. Each panel has reaction cavities molded into each individual plastic disposable tray. Reaction cavities contain dehydrated reactants which allow the simultaneous inoculation of each cavity with a predetermined amount of inoculum. A suspension of the test organism in RapID Inoculation Fluid is used as the inoculum. This is used to rehydrate and initiate test reactions. After incubation of the panel each test cavity is examined for reactivity by noting the development of a color. In some cases, reagents must be added to the test cavities to provide a color change. The resulting pattern of positive and negative test scores is used as the basis for identification by comparison of test results to reactivity patterns stored in a database or through the use of a computer-generated Code Compendium.

**SPECIMEN AND HANDLING:**

1. Test organisms must be grown anaerobically in pure culture.
2. A Gram stain must be performed and recorded for later use in the identification procedure.
3. Test organisms may be removed from a variety of selective and nonselective agar media. Recommended media include:
* Non-Selective Media: 5-7% Sheep Blood Agar prepared with Brucella, Columbia, Brain Heart Infusion, Lombard-Dowell, or Tryptic Soy base; CDC Anaerobic Blood Agar
* Differential/Selective Media: Phenylethyl Alcohol (PEA) Agar; Egg Yolk (EYA) Agar; Paromomycin/Vancomycin (PV) Agar; Kanamycin/Vancomycin (KV) Agar

## Materials Provided

20 RapID™ ANA II Panels 2 Chipboard Incubation Trays

20 Report Forms Instructions for Use

1 Dropper-Bottle RapID™ ANA II Reagent

### Materials Required But Not Provided

RapID™ Inoculation Fluid (1 ml) #3 McFarland Standard

RapID™ Spot Indole Reagent Cotton Swabs

RapID™ ANA II Code Compendium Microscope and Slides

or ERIC® Pipettes

Gram Stain Reagents Incubator (35-37°C, non-CO2)

**Storage Requirements**

 Store at 2-8°C in the original container.

## Product Deterioration

The expiration date and storage conditions for RapID™ ANA II System components are stated on the outer package. All materials will retain their reactivity for the period indicated if stored as directed.

**QUALITY CONTROL:**

RapID ANA II System Panel and reagent quality control are performed with each lot number and shipment of panels. The following table lists expected results for the selected test organisms, including those recommended as key indicators strains for streamlined QC as indicated in the CLSI document, M50-A.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Before Reagent Addition** | **After RapID™ ANA II Reagent Addition** | **Spot Indole** |
| **Organism** | **URE** | **BLTS** | **αARA** | **ONPG** | **αGLU** | **βGLU** | **αGAL** | **αFUC** | **NAG** | **PO4** | **LGY** | **GLY** | **PRO** | **PAL** | **ARG** | **SER** | **PYR** | **IND** |
| ***Clostridium sordellii*a****ATCC® 9714** | + | – | – | – | – | – | – | V | – | – | – | V | + | V | V | V | V | + |
| ***Bacteroides distasonis*a****ATCC® 8503** | – | + | V | + | + | + | + | – | + | + | + | + | – | + | + | + | + | – |
| *Bacteroides uniformis* ATCC® 8492 | – | + | + | + | + | + | + | + | + | + | + | – | – | – | – | – | – | + |
| +, positive; –, negative; V, variable |
| aKey indicator strains demonstrate acceptable performance of the most labile substrate in the system and reactivity in a significant number of wells, according to Clinical and Laboratory Standards Institute (CLSI) recommendations for streamlined quality control.1 |

**PROCEDURE:**

1. Panel Preparation:

Remove the RapID™ ANA II panel from storage. Do not use if the plastic tray is broken or if the lid is compromised. Reseal the plastic package after removing the required panels and chipboard incubation trays. Panels must be used the same day they are removed from storage.

1. Inoculum Preparation:

Using a cotton swab or an inoculating loop, remove sufficient growth from the agar plate culture to prepare a suspension of the test organism in RapID™ Inoculation Fluid that is EQUIVALENT to a #3 McFarland standard.

**NOTES:**

* Suspensions slightly more turbid than a #3 McFarland standard will not compromise test performance and are recommended for stock cultures and quality control strains.
* Suspensions prepared with a turbidity far less than or far greater than a #3 McFarland standard will compromise test performance.
* Suspensions should be thoroughly mixed and introduced into panels within fifteen (15) minutes of preparation.

An agar plate may be inoculated for purity or for any additional testing that may be required, using a loopful of test organism suspension. Incubate the plate for 18-24 hours at 35-37°C in an anaerobic atmosphere.

1. Panel Inoculation:

Peel back the lid over the RapID™ ANA II Panel inoculation port by pulling the tab marked “Peel to Inoculate” up and to the left. Using a pipette, transfer the entire inoculum (1ml) into the upper right hand corner of the panel.

Keeping the panel on a level surface, tilt back to approximately a forty-five (45) degree angle and rock from side to side to evenly distribute the inoculum along the rear baffles.

Maintaining a level, horizontal position, SLOWLY tilt the panel forward. The inoculum will flow from the rear baffles along the partitions into the front reaction cavities.

NOTES:

* Examine the test panel after inoculation to ensure that all cavities appear uniformly filled and bubble-free. Slight irregularities in cavity fill are acceptable. Discard any panels that contain GROSSLY misfilled cavities and repeat the inoculation procedure.
* Complete the inoculation of each panel receiving inoculation fluid before inoculating additional panels.
* Do not allow the inoculum to rest in the back portion of the panel for prolonged periods without completing the procedure.
	1. Incubation:

Place the inoculated panels in the chipboard incubation tray provided in the kit package and incubate in an aerobic (non-CO2) incubator at 35-37°C for four (4) hours, but no more than six (6) hours.

NOTE: If desired, after an incubation of 4-6 hours, and prior to the addition of any reagents, the RapID™ ANA II Panels may be placed in the refrigerator (2-8°C) overnight for reading the following morning.

**REPORTING RESULTS**

1. Remove panels from the incubation tray.
2. While firmly holding the RapID™ ANA II Panel on the benchtop, peel back the lid over the reaction cavities by pulling the lower right hand tab up and to the left.
3. Without the addition of any reagents, read and score cavities 1 (URE) through 10 (PO4) using the interpretation guide below. Record results in the appropriate boxes on the report form.

Cavities 1-10 contain the following tests:

Test: URE BLTS αARA ONPG αGLU βGLU αGAL αFUC NAG PO4

Cavity: 1 2 3 4 5 6 7 8 9 10

Test interpretation:

 Positive Negative

* + 1. Cavity 1 (URE) Red or Purple Yellow to Orange
		2. Cavities 2-10: Medium or Clear, Tan, or Very

(BLTS, αARA, Bright Yellow Pale Yellow

 ONPG, αGLU,

βGLU, αGAL,

αFUC, NAG, PO4)

1. Cavities 3-10 are bifunctional, containing two separate tests in the same cavity. After reading and recording the results for cavities 1-10, add the following reagents to the cavities indicated.
	1. Add 2 drops of RapID™ Spot Indole Reagent to cavity 10 (IND). **NOTE:** Only RapID™ Spot Indole Reagent should be used. Kovac’s or Ehrlich’s indole reagent will not provide satisfactory results.
2. Add 2 drops of RapID™ ANA II Reagent to cavities 3 (LGY) through 9 (PYR).Allow at least 30 seconds but no longer than 2 minutes for color development. Read cavities 3-10 and record results in the appropriate boxes on the report form.
	1. Cavities 3-10 contain the following tests after reagent addition:

Test: LGY GLY PRO PAL ARG SER PYR IND

Cavity: 3 4 5 6 7 8 9 10

* 1. Test interpretation after reagent addition:

 Positive Negative

Cavities 3-9: Purple, Violet Yellow, Orange

 (LGY, GLY, PRO, Red or Dark Pink or Pale Pink

 PAL, ARG, SER, PYR)

Cavity 10 (IND): Blue or Blue-Green Any other color

A 6 digit RapID™ ANA II microcode number is derived from the results recorded on the RapID™ ANA II report form. Refer to the RapID™ ANA II Code Compendium introduction for an explanation of the procedure for developing RapID™ ANA II microcodes. Reference the microcode obtained from the report pad in the RapID™ ANA II Code Compendium or ERIC® for the identification. If the microcode is not found in either of these sources, contact Technical Services at 1-800-255-6730.

**Limitations:**

1. The use of the RapID ANA II System and the interpretation of results require a competent laboratorian who is trained in general microbiological methods and who judiciously makes use of experience, knowledge, specimen information, and or other pertinent procedures before reporting the identity of isolates.
2. The accuracy of RapID System is based upon the correct use of laboratory procedures such as aerotolerance, gram stain, and growth characteristics
3. The use of mixed microbial population or direct testing of clinical material without culture will result in aberrant results.
4. The accuracy of the RapId ANA II System is based upon the statistical use of a multiplicity of specially designed tests and an exclusive, proprietary database. The use of any single test in the system to establish the identification of a test is subject to the error inherent in that test alone.

**REFERENCES:**

1. RapID ANA II System package Insert REF 8311002 REMEL Inc., Norcross, GA
2. RapID ANA II System Electronic RapID Compendium ERIC, REMEL Inc., Norcross, GA
3. Clinical and Laboratory Standards Institute. 2008. Quality Control for Commercial Microbial Identification Systems; Approved Guideline. M50-A. CLSI, Wayne, PA.