

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY TEMPLATE**

**A. 510(k) Number:**

K132491

**B. Purpose for Submission:**

To obtain substantial equivalence for Mueller-Hinton Agar Medium

**C. Measurand:**

Antimicrobial Susceptibility Test (Disk Diffusion)

**D. Type of Test:**

Culture Medium for Antimicrobial Susceptibility Test (Agar)

**E. Applicant:**

Edge Biologicals, Incorporated

**F. Proprietary and Established Names:**

Mueller Hinton Agar

**G. Regulatory Information:**

1. Regulation section:

866.1700

2. Classification:

II

3. Product code:

JTZ – Culture Media, Antimicrobial Susceptibility Test, Mueller Hinton Agar/Broth

4. Panel:

83 Microbiology

## **H. Intended Use:**

1. Intended use(s):

Mueller Hinton Agar is a standard basal medium intended for *in vitro* antimicrobial disk diffusion susceptibility testing of isolated colonies of common, rapidly growing bacteria by the Bauer-Kirby method as standardized by the Clinical and Laboratory Standards Institute (CLSI). This product has not been evaluated for gradient diffusion testing.

2. Indication(s) for use:

Same as above

3. Special conditions for use statement(s):

Prescription Use

4. Special instrument requirements:

Not Applicable

## **I. Device Description:**

Mueller Hinton agar is a standard basal medium recommended by the CLSI for routine antimicrobial disk diffusion susceptibility testing of rapidly growing (non-fastidious) bacteria. Beef and casein extracts and soluble starch in an agar base make up the nutritive base of the medium. Starch protects the test organism from toxic materials that may be in the medium. Calcium, magnesium, thymine, and thymidine concentrations are controlled.

## **J. Substantial Equivalence Information:**

1. Predicate device name(s):

Mueller Hinton Agar with 5% Sheep Blood

2. Predicate 510(k) number(s):

K960420

3. Comparison with predicate:

**Table 1 - Similarities and Differences of Mueller Hinton Agar and the Predicate**

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	<b>Mueller Hinton Agar (Edge Biologicals, Inc.)</b>	<b>Mueller Hinton Agar with 5% Sheep Blood – K960420 (Becton Dickinson)</b>
Intended Use	Disk diffusion antimicrobial susceptibility testing	Same
Source of Microorganisms for Testing	Bacterial colonies isolate from culture	Same
Inoculum	0.5 McFarland Suspension, streaked over entire plate	Same
Read Method	Measure zones of inhibition in millimeters	Same
Storage	2 - 8 degrees Celsius	Same

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Formulation	Beef Extract, Acid Hydrolysate of Casein, Starch, Agar	Beef Extract, Acid Hydrolysate of Casein, Starch, Agar, 5% Sheep Blood Agar
Test Microorganisms	Common, rapidly growing, non-fastidious microorganisms	Fastidious microorganisms

**K. Standard/Guidance Document Referenced (if applicable):**

- CLSI M02-A11, Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – Eleventh Edition
- CLSI M100-S24, Performance Standards for Antimicrobial Susceptibility Testing; Twenty Fourth Informational Supplement

**L. Test Principle:**

In the early 1960's Bauer, Kirby and others developed a standardized procedure in which Mueller Hinton Agar, a medium originally devised for the isolation of gonococci, was selected as the test medium for determining the susceptibility of bacteria to antibiotic and chemotherapeutic agents. A subsequent international collaborative study confirmed the value of Mueller Hinton Agar for this purpose because of the relatively good reproducibility of the medium, the simplicity of its formula, and the wealth of experimental data that had been accumulated using this medium.

The Bauer-Kirby procedure is based on the diffusion through agar medium (such as

Mueller Hinton agar) of antimicrobial substances which are impregnated on sterile paper disks. This method employs disks with a single concentration of antimicrobial agent and zone sizes are correlated with minimum inhibitory concentrations. In the test procedure, a standardized suspension of the organism is swabbed over the entire surface of the agar medium. Sterile paper disks impregnated with specified amounts of antibiotic or other antimicrobial agents are then placed on the surface of the inoculated agar medium. The agar medium is incubated at  $35 \pm 2^\circ\text{C}$  for 16-18 hours. The organism will grow as a solid "lawn". The antimicrobial will diffuse outward (in a circle). If the antimicrobial agent has activity against the organism, a circular zone of growth inhibition will result. The zone of inhibition around the paper disk is measured. A determination as to whether the organism is susceptible, intermediate or resistant to the antimicrobial agent is determined by comparing the size of the zone of inhibition to the zone diameter interpretive criteria in the CLSI M100 Standard. There are various factors which can influence the disk diffusion susceptibility testing such as the agar medium, excess surface moisture on the medium, agar depth, antimicrobial disk potency, inoculum concentration, and medium pH.

#### **M. Performance Characteristics (if/when applicable):**

##### 1. Analytical performance:

###### *a. Precision/Reproducibility:*

The performance of Mueller Hinton Agar was evaluated in a multi-site reproducibility study. Antimicrobial disk diffusion susceptibility testing was conducted at three sites. Two of the test sites were external and one test site was internal. Six organisms representing Gram negative and Gram positive rapidly growing (non-fastidious) CLSI recommended quality control strains from American Type and Culture Collection (ATCC) were included in the study, namely, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *E. coli* 25922, *E. coli* ATCC 35218 and *P. aeruginosa* ATCC 27853. Antimicrobials representing the major drug classes relevant to each organism were included in the study. Testing was conducted on three separate days, in duplicate, using two different lots of Mueller Hinton Agar, two different plate sizes (15 X 90 mm and 15 X1 50 mm), and where available, antimicrobial disks from two different manufacturers resulting in a total of 72 results for each organism-antimicrobial combination. Antimicrobial disks from only one manufacturer were available at the time of testing for Cefepime, Ceftaroline, High Level Gentamicin, and High Level Streptomycin, resulting in a total of 36 results for each of these antimicrobials.

The performance of Mueller Hinton Agar was evaluated relative to the acceptable range as indicated in the antimicrobial specific FDA drug label, or CLSI Standards M100-S24 or M02-A11. Acceptable performance for each organism-antimicrobial combination was determined as test results falling within the acceptable range  $\geq 95\%$  of the time.

Levels of thymine and thymidine in Mueller Hinton Agar were evaluated by testing *Enterococcus faecalis* ATCC 29212 with trimethoprim/sulfamethoxazole. Excess amounts of thymine and thymidine can reverse the inhibitory effect of sulfonamides and trimethoprim, resulting in smaller, less distinct zones of inhibition or no zones at all. The results of the testing of this organism-antimicrobial combination were within range, indicating adequate levels of thymine and thymidine.

The levels of the cations calcium and magnesium in Mueller Hinton Agar were evaluated by testing *Pseudomonas aeruginosa* ATCC 27853 with the aminoglycosides gentamicin and tobramycin. Excess cation content results in reduced zones, whereas low cation content results in unacceptably large zone sizes. The results of the testing of these antimicrobials were within range, indicating adequate levels of calcium and magnesium.

The results of the study for each organism-antimicrobial combination were within acceptable range  $\geq 95\%$  of the time.

Reproducibility study results for Mueller Hinton Agar are represented in Tables 2 through 7 below.

**Table 2 – Reproducibility Study Results - *E. coli* ATCC 25922**

Item No.	Name of Antimicrobial	Total number of Results within acceptable range/ Total number of test results	% of Results Within Acceptable Range
1	Ampicillin/Sulbactam	71/72	98.6
2	Ampicillin	72/72	100
3	Cefazolin	72/72	100
4	Cefepime	35/36*	97.2
5	Cefotaxime	72/72	100
6	Cefoxitin	71/72	98.6
7	Cefuroxime	72/72	100
8	Gentamicin	72/72	100
9	Imipenem	72/72	100
1	Levofloxacin	72/72	100
11	Nitrofurantoin	72/72	100
12	Piperacillin	72/72	100
13	Tobramycin	72/72	100
14	Trimethoprim/ Sulfamethoxazole	72/72	100

\*Antibiotic disks from only one manufacturer were available at the time of testing.

**Table 3 – Reproducibility Study Results - *E. coli* ATCC 35218**

Item No.	Name of Antimicrobial	Total number of Results within acceptable range/ Total number of test results	% of Results Within Acceptable Range
1	Ampicillin/Sulbactam	72/72	100
2	Amoxicillin/Clavulanic Acid	72/72	100
3	Ampicillin	72/72	100
4	Piperacillin/Tazobactam	72/72	100
5	Ticarcillin/Clavulanic Acid	72/72	100

**Table 4 – Reproducibility Study Results - *E. faecalis* ATCC 51299**

Item No.	Name of Antimicrobial	Total number of Results within acceptable range / Total number of test results	% of Results Within Acceptable Range
1	Vancomycin**	72/72	100

\*\* Test plates were incubated for a full 24 hours prior to reading and interpretation.

**Table 5 – Reproducibility Study Results - *E. faecalis* ATCC 29212**

\* Antibiotic disks from only one manufacturer were available at the time of testing

Item No.	Name of Antimicrobial	Total number of Results within acceptable range / Total number of test results	% of Results Within Acceptable Range
1	High Level Gentamicin	36/36*	100
2	High level Streptomycin	36/36*	100
3	Trimethoprim/ Sulfamethoxazole	72/72	100

**Table 6 – Reproducibility Study Results - *Staphylococcus aureus* ATCC 25923**

Item No.	Name of Antimicrobial	Total number of Results within acceptable range/ Total number of test results	% of Results Within Acceptable Range
1	Erythromycin	72/72	100
2	Cefepime	36/36*	100
3	Cefoxitin	69/72	95.8
4	Ceftaroline	36/36*	100
5	Clindamycin	72/72	100
6	Oxacillin	72/72	100
7	Tetracycline	72/72	100
8	Tobramycin	72/72	100
9	Trimethoprim/ Sulfamethoxazole	71/72	98.6
10	Vancomycin**	72/72	100

\* Antibiotic disks from only one manufacturer were available at the time of testing.

\*\* Test plates were incubated for a full 24 hours prior to reading and interpretation.

**Table 7 – Reproducibility Study Results - *Pseudomonas aeruginosa* ATCC 27853**

Item No.	Name of Antimicrobial	Total number of Results within acceptable range/ Total number of test results	% of Results Within Acceptable Range
1	Cefepime	36/36*	100
2	Ceftazidime	72/72	100
3	Gentamicin	72/72	100
4	Imipenem	72/72	100
5	Levofloxacin	72/72	100
6	Piperacillin	72/72	100
7	Tobramycin	71/72	98.6

\* Antibiotic disks from only one manufacturer were available at the time of testing

b. *Linearity/assay reportable range:*

Not Applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Six organisms representing Gram negative and Gram positive rapidly growing (non-fastidious) CLSI recommended quality control strains from American Type and Culture Collection (ATCC) were included in the reproducibility study, namely, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *E. coli* 25922, *E. coli* ATCC 35218 and *P. aeruginosa* ATCC 27853. Antimicrobials

representing the major drug classes relevant to each quality control strain were tested. The results of the study for each organism-antimicrobial combination were within acceptable range  $\geq 95\%$  of the time.

*d. Detection limit:*

Not Applicable

*e. Analytical specificity:*

Not Applicable

*f. Assay cut-off:*

Not Applicable

2. Comparison studies:

*a. Method comparison with predicate device:*

Device performance was evaluated via the conduct of a multi-site reproducibility study. See Section M (1)(a) above for a detail description.

*b. Matrix comparison:*

Not Applicable

3. Clinical studies:

*a. Clinical Sensitivity:*

Not Applicable

*b. Clinical specificity:*

Not Applicable

*c. Other clinical supportive data (when a. and b. are not applicable):*

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

The acceptable zone diameter ranges for various antimicrobial agents are listed in Table 8 below for the six CLSI recommended quality control strains used in the Mueller Hinton Agar performance testing.

**Table 8 – Acceptable Zone Diameter Ranges for each antimicrobial agent/QC strain tested**

Item No.	Name of Antimicrobial and disk potency in micrograms (µg)	Acceptable Zone Range in millimeters (mm)
<b><i>E. coli</i> 25922</b>		
1	Ampicillin/Sulbactam (10/10µg)	19-24 mm
2	Ampicillin (10µg)	16-22mm
3	Cefazolin (30µg)	21-27mm
4	Cefepime (30µg)	31-37mm
5	Cefotaxime (30µg)	29-35mm
6	Cefoxitin (30µg)	23-29mm
7	Cefuroxime (30µg)	20-26mm
8	Gentamicin (10µg)	19-26mm
9	Imipenem(10µg)	26-32mm
1	Levofloxacin (5µg)	29-37mm
11	Nitrofurantoin (300µg)	20-25mm
12	Piperacillin(100µg)	24-30mm
13	Tobramycin (10µg)	18-26mm
14	Trimethoprim/ Sulfamethoxazole (1.25/23.75µg)	23-29mm
<b><i>E. coli</i> 35218</b>		
1	Ampicillin/Sulbactam (10/10µg)	13-19mm
2	Amoxicillin/Clavulanic Acid (20/10 µg)	17-22mm
3	Ampicillin (10µg)	6 mm
4	Piperacillin/Tazobactam (100/10µg)	24-30mm
	Ticarcillin/Clavulanic Acid (75/10µg)	21-25mm
<b><i>E. faecalis</i> 51299</b>		
1	Vancomycin (30µg)	≤14mm
<b><i>E. faecalis</i> 29212</b>		
1	High Level Gentamicin (120µg)	16-23mm
2	High Level Streptomycin (300µg)	14-20mm
3	Trimethoprim/ Sulfamethoxazole (1.25/23.75µg)	>20mm

<i>S. aureus</i> 25923		
1	Erythromycin (15µg)	22-30mm
2	Cefepime (30µg)	23-29mm
3	Cefoxitin (30µg)	23-29mm
4	Ceftaroline (30µg)	26-35mm
Item No.	Name of Antimicrobial	Acceptable Range
5	Clindamycin (2µg)	24-30mm
6	Oxacillin (1µg)	18-24mm
7	Tetracycline (30µg)	24-30mm
8	Tobramycin (10µg)	19-29mm
9	Trimethoprim/ Sulfamethoxazole (1.25/23.75µg)	24-32mm
10	Vancomycin (30µg)	17-21mm
<i>P. aeruginosa</i> 27853		
1	Cefepime (30µg)	24-30mm
2	Ceftazidime (30µg)	22-29mm
3	Gentamicin (10µg)	17-23mm
4	Imipenem (10µg)	20-28mm
5	Levofloxacin (5µg)	19-26mm
6	Piperacillin (100µg)	25-33mm
7	Tobramycin (10µg)	19-25mm

**N. Proposed Labeling:**

The device labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.