IFU

Instructions for Use

BLUECOLI™ URINE BIPLATE

Cat. no. J123	BluEcoli™ Urine Biplate, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

Hardy Diagnostics BluEcoli™ Urine Biplate is a urine culture media, consisting of Blood Agar on one side and BluEcoli™ Agar on the other side, which is used for the isolation of urinary pathogens and for the identification of *E. coli*.

SUMMARY

Diagnosis of urinary tract infections contributes significantly to the daily workload in a microbiology laboratory, therefore any attempts of innovation by reducing the work load and cost are always welcome while a high quality standard is still maintained. For several years, development of culture media containing chromogens and fluorogens has led to the development of a great number of methods for the identification of microorganisms in primary isolation media.

The BluEcoli™ Agar side of the biplate uses a MacConkey Agar base with beta-D-glucoronidase (GUD) as indicator for *E. coli* since this enzyme is present in 94-96% of members of this species.[1] Presence of GUD can be measured by using different chromogenic and fluorogenic substrates. Fluorogens are hydrolyzed by GUD yielding fluorescent colonies of *E. coli* under a long-wave (366nm) UV light source, whereas chromogens release chromophores resulting in easy to read purple-blue colored colonies of *E. coli*.[1]

E. coli is known as the most common pathogen responsible for great majority (75-90%) of urinary tract infections.[11] Therefore rapid identification and reporting is useful in the direction of therapy, and also streamlines the overall turn-around-time and allows microbiologists to devote more time to problematic cultures.[2,3]

Several chromogenic media have been compared to traditional urine culture media (i.e., Blood and MacConkey Agars) and were found to be at least as good as traditional media for the isolation of urinary pathogens.[2-5] Overall, studies agree that rapid detection and identification of microorganisms is of high importance in a diverse array of clinical and research settings. By incorporating synthetic enzyme substrates into primary isolation media, the enumeration and detection by color reactions can be performed directly on the isolation plate. This enhances the accuracy and performance of the microbiologist, allowing them to quickly recognize *E. coli* isolates and rule out clinically insignificant mixed cultures.[3]

Since very limited information is documented, in terms of accuracy, concerning usage of colonies from chromogenic media for susceptibility testing in automated systems, it is recommended that colonies from the Blood Agar side of the BluEcoli™ Urine Biplate be used for susceptibility testing.

FORMULA

Ingredients per liter of deionized water:*

Blood Agar (Side I):	
Pancreatic Digest of Casein	15.0gm
Pancreatic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Sheep Blood	50.0ml
Agar	12.0gm

Final pH 7.3 +/- 0.2 at 25ºC.

BluEcoli™ Agar (Side II):	
Pancreatic Digest of Gelatin	17.0gm
Lactose	10.0gm
Sodium Chloride	5.0gm
Pancreatic Digest of Casein	1.5gm
Peptic Digest of Animal Tissue	1.5gm
Bile Salts	1.5gm
Neutral Red	30.0mg
Chromogenic Substances	90.0mg
Crystal Violet	1.0mg
Agar	13.5gm

Final pH 7.1 +/- 0.3 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be

considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Specimens should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be contained within an air-tight transport vial and refrigerated until inoculation. Consult listed references for information on specimen collection.(6-10)

Prepared media should be inoculated, incubated, and results recorded according to accepted procedures described in the listed reference texts.(6-10)

INTERPRETATION OF RESULTS

E. coli appears as purple-blue colored colonies on the BluEcoli™ Agar side of the biplate. No further testing is needed.

Other gram-negative rods that grow on the BluEcoli™ Agar side may appear pink to slight pink for lactose-positive colonies, and colorless for lactose-negative colonies, as is typical of MacConkey Agar.

The Blood Agar side of the biplate will exhibit typical colony morphology of urinary pathogens and contaminating organisms.

LIMITATIONS

For organisms other than *E. coli*, biochemical, immunological, molecular, or mass spectrometry testing should be performed on colonies from pure culture for complete identification

Speciation and susceptibility tests should be done with pure cultures taken from the Blood Agar side of the biplate. Erroneous antimicrobial susceptibility results may occur from colonies taken from the BluEcoli™ Agar side.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as calibrated loops, specimen cups, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Blood Agar (Side I):					

Streptococcus pneumoniae ATCC® 6305	A	24hr	35°C	Aerobic	Growth; alpha-hemolysis
Streptococcus pyogenes ATCC® 19615**	A	24hr	35°C	Aerobic	Growth; beta-hemolysis
Staphylococcus aureus ATCC® 25923	A	24hr	35°C	Aerobic	Growth
Escherichia coli ATCC® 25922	A	24hr	35°C	Aerobic	Growth
CAMP Test:					
Test Organisms	**Inoculation Method***	**Incubation**			**Results**
		Time	**Temperature**	**Atmosphere**	
Staphylococcus aureus ATCC® 33862	H	18-24hr	35°C	Aerobic	Growth
Streptococcus agalactiae ATCC® 12386	H	18-24hr	35°C	Aerobic	Growth; positive (enhanced arrowhead hemolysis)
Streptococcus pyogenes ATCC® 19615	H	18-24hr	35°C	Aerobic	Growth; negative (no enhanced arrowhead hemolysis)
BluEcoli™ Agar (Side II):					
Test Organisms	**Inoculation Method***	**Incubation**			**Results**
		Time	**Temperature**	**Atmosphere**	
Escherichia coli ATCC® 25922**	A	24hr	35°C	Aerobic	Growth; purple-blue colonies
Enterobacter cloacae ATCC® 23355	A	24hr	35°C	Aerobic	Growth; pink colonies
Klebsiella pneumoniae ATCC® 13883**	A	24hr	35°C	Aerobic	Growth; pink colonies
Proteus mirabilis ATCC® 12453	A	24hr	35°C	Aerobic	Growth; clear colonies
Staphylococcus aureus ATCC® 25923	B	24hr	35°C	Aerobic	Inhibited
Enterococcus faecalis ATCC® 29212	B	24hr	35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or

a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

BluEcoli™ Urine Biplate should appear as follows:

Blood Agar (Side I) should appear opaque, and cherry red in color.
BluEcoli™ Agar (Side II) should appear translucent, and pink in color.

Escherichia coli (ATCC® 25922) colonies growing on a BluEcoli™ Urine Biplate (Cat no. J123). Incubated aerobically for 24 hours at 35ºC.

Mixed culture with *Enterobacter cloacae* (ATCC® 23355) colonies (pink) and *Escherichia coli* (ATCC® 25922) colonies (blue) growing on BluEcoli™ Agar (Cat no. J123). Incubated aerobically for 24 hours at 35ºC.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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