IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

ENTEROSCREEN 4™

Cat. no. L225	EnteroScreen 4™, 16x125mm Tube, 4 Layer Slant, 12ml	20 tubes/box

INTENDED USE

Hardy Diagnostics EnteroScreen 4™ is recommended for screening of stool specimen isolates of non-lactose-fermenting, oxidase-negative, enteric pathogens and the differentiation of *Salmonella* and *Shigella* to rule out normal enteric flora.

SUMMARY

Three media, Triple Sugar Iron (TSI) Agar, Lysine Iron Agar (LIA), and Urea Agar, are used to screen non-lactose-fermentative cultures isolated from stool specimens. This battery of tests is helpful in differentiating *Salmonella* and *Shigella* from other members of the Enterobacteriaceae family.[2,3,5] Hardy Diagnostics EnteroScreen 4™ contains a unique media combination, which has been demonstrated to be less redundant and labor intensive, yet equivalent in performance, to the traditional three tube set. It is recommended that EnteroScreen 4™ be used in conjunction with the developed algorithm (see Figure 2 or Figure 3) for organism identification. The EnteroScreen 4™ system consists of four layers in a single tube; three agar layers and a petrolatum layer (see Figure 1). The tube contains, in order from bottom to top, Urea Agar, Petrolatum Plug, Modified Lysine Iron Agar, and Lysine Iron Agar. With this method, urease activity, lysine-decarboxylase, lysine-deaminase, gas production, and hydrogen sulfide production reactions can be simultaneously evaluated.[6,7]

Urea Agar, the bottom agar layer, contains urea and phenol red as a pH indicator. Organisms capable of hydrolyzing urea form ammonia as a by-product, thus turning the medium alkaline. The pH indicator turns from pale orange to pink-red in color under these conditions. The reduced buffer content and peptone in this medium promote more rapid growth and reaction time for many members of the Enterobacteriaceae. Dextrose is included in the formulation to stimulate urease activity in organisms that hydrolyze urea slowly, and to exclude false-negative reactions.

Urea Agar is added to the tube to differentiate other non-lactose-fermenters, i.e. *Proteus*, *Morganella* and *Providencia* species, from *Yersinia enterocolitica*, lactose-negative *E. coli*, *Edwardsiella*, *Salmonella*, and *Shigella* species. These latter organisms, all potential stool pathogens, can be separated from non-pathogenic organisms by the inability to hydrolyze urea. However, some strains of *Yersinia enterocolitica* may hydrolyze urea and can be differentiated from non-pathogenic organisms by the inability to deaminate lysine, lack of H_2S production, and the absence of gas. The Urea Agar, is separated from the next agar layer by the addition of a sterile, hydrophobic petrolatum plug.[2,3,5-7]

Modified Lysine Agar comprises the agar layer above the petrolatum plug and is used to detect by-products of dextrose-fermentation and the presence of lysine-decarboxylase. The media formulation is similar to traditional Lysine Iron Agar (LIA), however, hydrogen sulfide indicators are absent, to prevent excessive hydrogen sulfide production from masking the lysine-decarboxylase reaction.[6,7] The media contains 0.1% percent dextrose as a carbohydrate source. Enteric organisms that are capable of fermenting dextrose will produce acid and sometimes gas, seen as cracks and bubbles in the medium.

Modified Lysine Iron Agar is especially useful for differentiating organisms from the genus *Salmonella* and

Edwardsiella from *Shigella* spp. and *Yersinia enterocolitica*, as the lysine-decarboxylase reaction is significantly easier to interpret than the traditional LIA reaction. *Salmonella* and *Edwardsiella* species possess lysine-decarboxylase enzymes, which remove a molecule of carbon dioxide from lysine to form an alkaline reacting amine. The pH indicator in the media, bromcresol purple, turns the agar purple in the presence of these alkaline compounds. A yellow color, the result of dextrose-fermentation, is observed in the agar when lysine-decarboxylation does **not** occur, as is the case with *Shigella* and *Yersinia enterocolitica* species.[6,7]

The top layer (the slanted portion) of EnteroScreen 4™ contains Lysine Iron Agar (LIA) which is used to detect hydrogen sulfide production as well as the deamination of lysine. LIA contains sodium thiosulfate and ferrous sulfate, which are added to detect organisms that have the ability to liberate sulfur from sulfur containing compounds in the form of hydrogen sulfide. Production of hydrogen sulfide generates an insoluble, heavy metal sulfide, which produces a black precipitate in the medium. Another advantage of LIA is that certain enteric organisms, like *Proteus* and *Providencia* species, possess enzymes responsible for the oxidative-deamination of lysine.[5-7] If lysine is deaminated, in the presence of oxygen, a red color is observed on the slant. A negative lysine-deamination reaction, observed when the slant is purple in color, is indicative of *Salmonella*, *Edwardsiella*, *Yersinia enterocolitica*, and *Shigella* species. Furthermore, organisms can be isolated from the slant, and used to perform additional biochemical and serologic testing.[6,7]

FORMULA[6]

Formulations are listed in order from the bottom (butt) of the tube to the top (slant).

Bottom Agar Layer:	
Urea Agar, 2ml/tube	
Urea	20.0gm
Sodium Chloride	5.0gm
Monopotassium Phosphate	2.0gm
Peptone	1.0gm
Yeast Extract	1.0gm
Dextrose	1.0gm
Phenol Red	12.0mg
Agar	12.0gm

Hydrophobic Layer:
Sterilized Petrolatum Plug, 2.5ml/tube

Middle Agar Layer:	
Modified Lysine Iron Agar (LIA), 2.5ml/tube	
L-Lysine	10.0gm
Sodium Chloride	5.0gm
Peptone	5.0gm
Yeast Extract	3.0gm
Dextrose	1.0gm

Bromcresol Purple	20.0mg
Agar	12.0gm

Top Agar Layer:	
Lysine Iron Agar (LIA), 5ml/tube (slant)	
L-Lysine	10.0gm
Sodium Chloride	5.0gm
Peptone	5.0gm
Yeast Extract	3.0gm
Dextrose	1.0gm
Ferrous Sulfate	0.3gm
Sodium Thiosulfate	0.3gm
Bromcresol Purple	20.0mg
Agar	12.0gm

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[6,7]

Specimen Collection: This product is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organism. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism.

The EnteroScreen 4™ algorithm is recommended for use only with non-lactose-fermenting, oxidase-negative organisms; the detection of oxidase-positive organisms will require the use of another test system (OxiStrips™, Cat. no. Z93).

Method of Use:

1. Allow the EnteroScreen 4™ tubes to warm to room temperature prior to use. In addition, it is recommended that a Blood Agar plate (Cat. no. A10) be run in parallel as a check of pure culture, as well as additional serology and biochemical testing.

2. Using an inoculation needle, obtain a well-isolated, lactose-negative colony from a differential media such as; MacConkey Agar (Cat. no. G35), HE Agar (Cat. no. G63), XLD Agar (Cat. no. G65), or SS Agar (Cat. no. G50).

3. Inoculate by stabbing to the bottom of the tube. Streak the surface of the slant while withdrawing the needle.

4. Incubate tubes aerobically with **loose caps** at 35-37ºC. Examine agar layers for reactions at 18-24 hours and again at 48 hours. Urea should not be read after 24 hours.

Note: An oxidase test (OxiStrips™, Cat. no. Z93) must be performed prior to interpreting the results of EnteroScreen 4™. See "Interpretation of Results" section for more information.

INTERPRETATION OF RESULTS[6]

Refer to Figure 2 and Figure 3 for algorithms describing expected reactions. Oxidase reactions obtained from the Blood Agar plate are useful in the EnteroScreen 4™ algorithm. Reactions are listed in order from bottom of tube to slant. See Table 1 (below) for more information.

Bottom Agar Layer - Urea Agar:
Urease Production Positive: pink color (alkaline reaction) Negative: light orange (acid reaction)

Hydrophobic Layer:
No reaction observed

Middle Agar Layer - Modified Lysine Iron Agar (LIA):
Lysine-Decarboxylase Positive: purple (alkaline reaction)* * Only a true purple color should be considered a positive reaction for lysine-decarboxylase. A purple-yellow color is a negative reaction. Negative: yellow (acid reaction) Gas Production as a Result of Dextrose-Fermentation Positive: presence of bubbles or cracks Negative: absence of bubbles or cracks

Top Agar Layer - Lysine Iron Agar (LIA):

Lysine-Deamination
Positive: red slant
Negative: purple or yellow

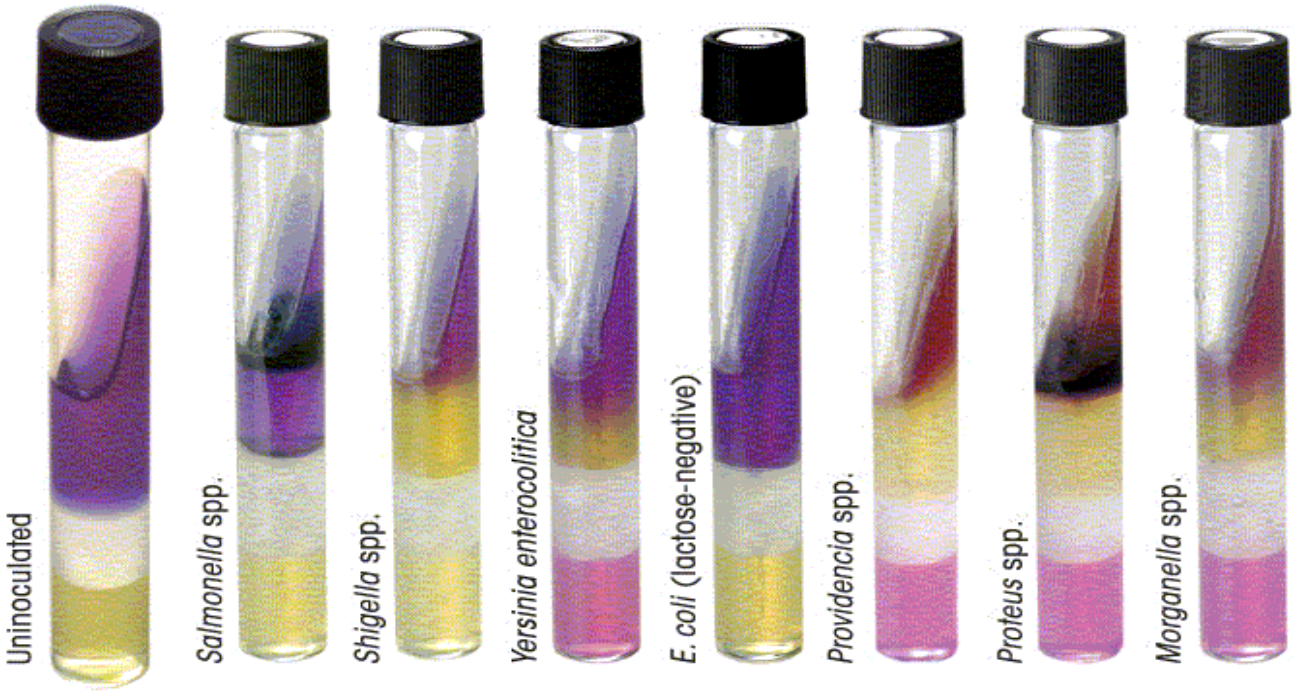
Hydrogen Sulfide Production
Positive: blackened medium, concentrated at the bottom of slant
Negative: purple bottom of slant

Typical Reactions of Lactose-Negative Enterobacteriaceae in EnteroScreen 4™ (Table 1)

Organism	**Urease**(Bottom Layer)	**Lysine-Decarboxylase**(Middle Layer)	**Lysine-Deaminase**(Slant)	**H_2S**	**Gas**	**PYR**	**Notes**
Salmonella spp.	orange (-)	purple/yellow (v)	purple (-)	black (+)	yes (+)	negative	Some *S. choleraesuis* and most *S. paratyphi* will be H_2S negative. All *Salmonella* spp. are lysine-decarboxylase positive except for *S. paratyphi*
E. coli (lactose-negative)	orange (-)	purple/yellow (v)	purple (-)	purple (-)	no (-)	negative	Similar to H_2S negative strains of *Edwardsiella* spp. but *E. coli* is ONPG and MUG positive.
Edwardsiella spp.	orange (-)	purple (+)	purple (-)	black/purple (v)	yes (+)	negative	H_2S negative strains similar to lactose-negative *E. coli* but *Edwardsiella* spp. is ONPG and MUG negative. H_2S positive strains similar to *Salmonella* spp. Confirm negative agglutination with *Salmonella* antisera. Most *Edwardsiella* are indole-positive and *Salmonella* spp. are indole-negative.
Yersinia enterocolitica	pink/orange (v)	yellow (-)	purple (-)	purple (-)	no (-)	positive	
Shigella spp.	orange (-)	yellow (-)	purple (-)	purple (-)	no (-)	negative	
Hafnia alvei	orange (-)	purple (+)	purple (-)	purple (-)	yes/no (v)	negative	
Providencia spp.	pink/orange (v)	yellow (-)	red (+)	red (-)	yes/no (v)	negative	
Proteus spp.	pink	purple/yellow	red	black/red	yes	negative	Most *P. penneri* will be

	(+)	(v)	(+)	(v)	(+)		H_2S negative.
Morganella spp.	pink (+)	purple/yellow (v)	red (+)	red/black (v)	yes (+)	negative	Most will be H_2S negative.
Citrobacter spp.	pink/orange (v)	yellow (-)	purple/yellow (-)	black/purple (v)	yes (+)	positive	Usually lactose-positive but rare lactose-negative exists.
Enterobacter spp.	pink/orange (v)	purple/yellow (v)	purple (-)	purple (-)	yes (+)	positive	Usually lactose-positive but rare lactose-negative exists.
Klebsiella spp.	pink/orange (v)	purple (+)	purple (-)	purple (-)	yes (+)	positive	Usually lactose-positive but rare lactose-negative exists.
Serratia spp.	orange (-)	purple/yellow (v)	purple (-)	purple (-)	yes/no (v)	positive	Usually lactose-positive but rare lactose-negative exists.

COLOR CODES:



Note: Some reactions are variable. See the EnteroScreen 4™ algorithm (Figure 2) for accurate interpretations.

FIGURES

Figure 1: Diagram illustrating components of Hardy Diagnostics EnteroScreen 4™.

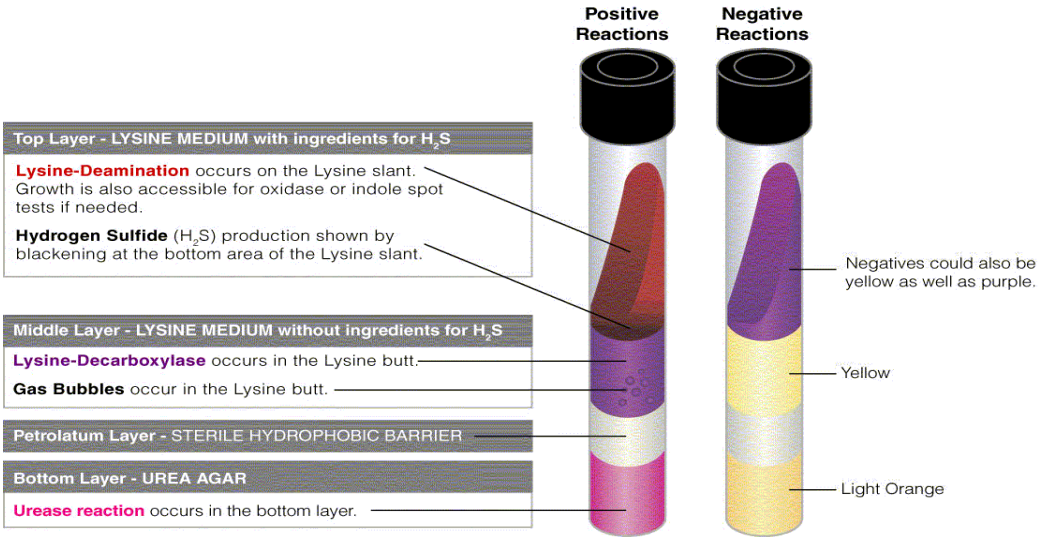


Figure 2: Algorithm for Hardy Diagnostics EnteroScreen 4™.[6]
The EnteroScreen 4™ algorithm is recommended for use with non-lactose-fermenting, oxidase-negative organisms only.

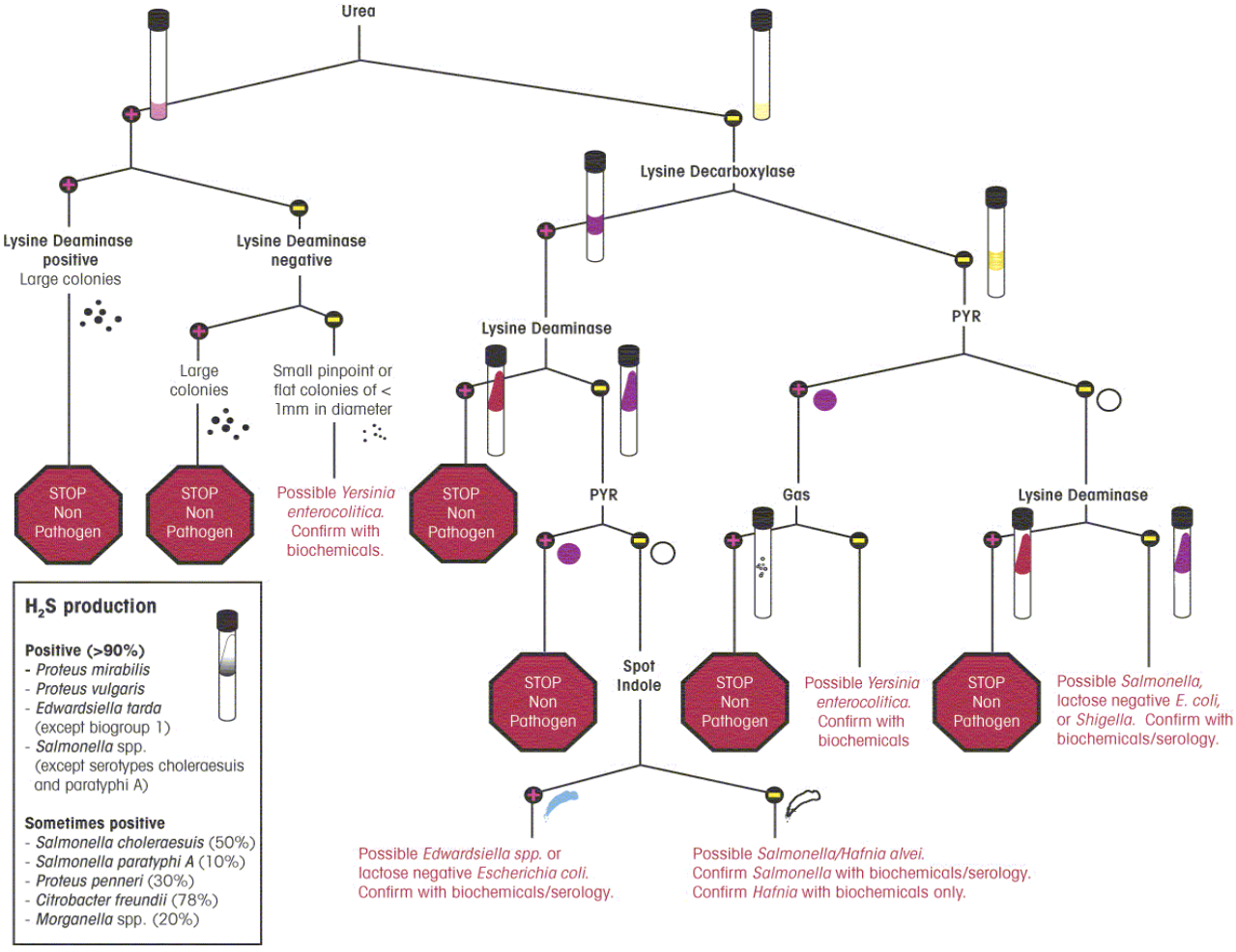
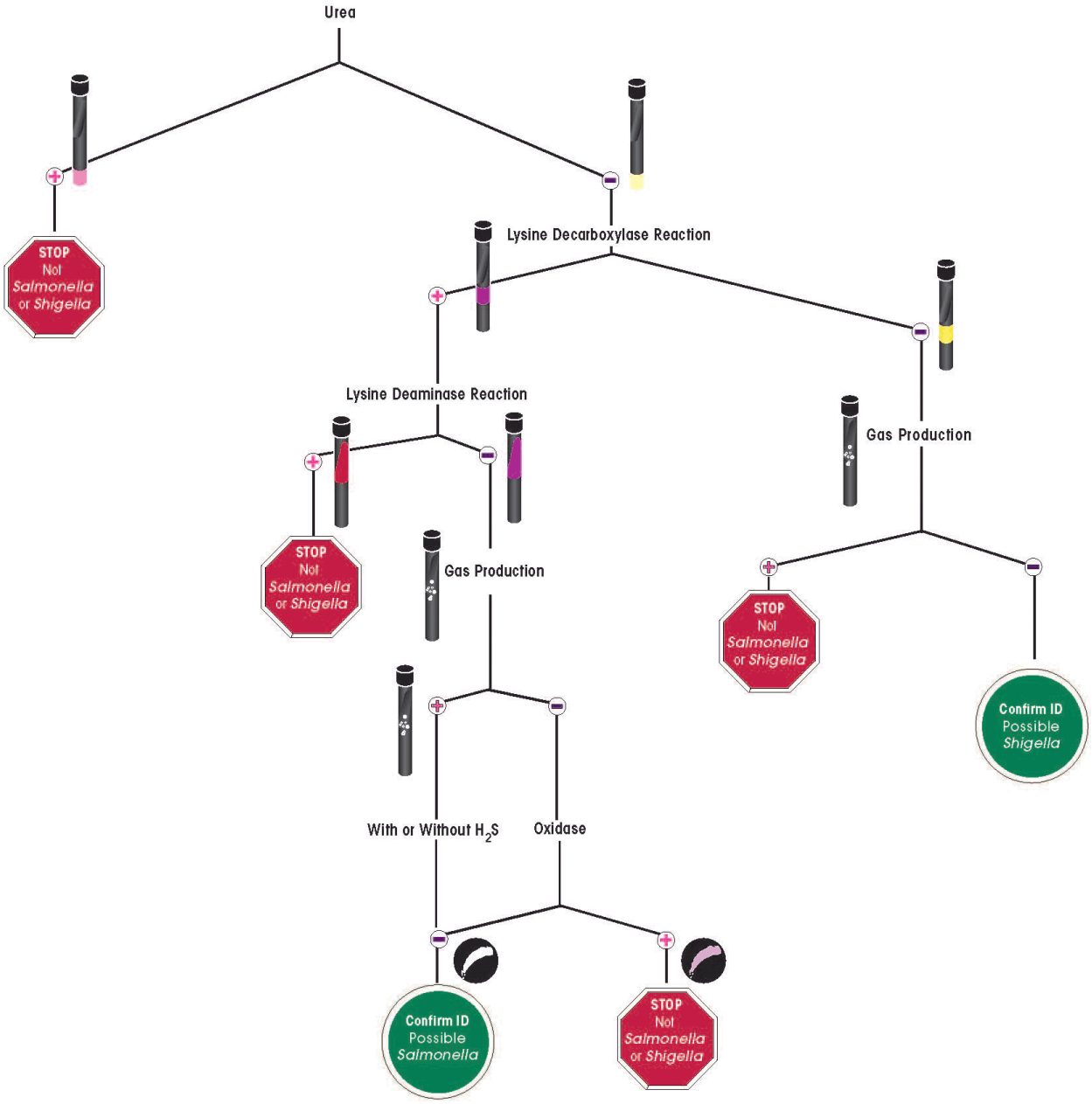


Figure 3: Abbreviated algorithm for Hardy Diagnostics EnteroScreen 4™.[7]
The EnteroScreen 4™ abbreviated algorithm is recommended for use with non-lactose-fermenting, oxidase-negative organisms only.



LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

It is important to stab the butt of the medium. Failure to stab all the way to the bottom (butt) of the media can produce weak or erroneous urease results.

Caps must be loosened during incubation or erroneous results will occur.

Certain species or strains may give delayed reactions or completely fail to ferment the carbohydrate in the stated manner. However, in most cases if the organism fails to ferment dextrose within 48 hours, and growth is definitely present, the organism is most likely not in the Enterobacteriaceae family and cannot be differentiated with the EnteroScreen 4™ algorithm. It is recommended that an oxidase test be performed prior to use with the EnteroScreen 4™ algorithm as a negative oxidase reaction will confirm that an organism is a member of Enterobacteriaceae.

After prolonged incubation a false-positive alkaline reaction may be seen in the Urea Agar. To rule out this occurrence, check the test with a control (an uninoculated tube of EnteroScreen 4™) along with the inoculated tube during

prolonged incubation.

Do not heat EnteroScreen 4™ above 37ºC., as urea decomposes very readily when heated.

Gastroenteritis has also been associated with *Edwardsiella tarda*, an organism that resembles *Salmonella* species in its pathogenic mechanisms, morphologically, as well as biochemically.[3] In addition to serology, an indole test (Cat. no. Z65) is useful in differentiating *Edwardsiella* (indole-positive) from *Salmonella* species (indole-negative). See EnteroScreen 4™ algorithm (Figure 2).

Hafnia alvei is a rare opportunistic stool pathogen which is biochemically similar to *Salmonella* species and agglutinates with *Salmonella* and *Shigella* antisera. Expected reactions for *Hafnia alvei* include urease-negative, lysine-decarboxylase-positive, and lysine-deaminase-negative. It can be differentiated from *Salmonella* by a positive VP reaction at 25ºC.

There are rare strains of *Citrobacter* that will show a reaction similar to *Salmonella paratyphi* (lactose-negative, urease-negative, H_2S-positive, gas-positive). These strains can be differentiated by a rapid PYR test (*Citrobacter* PYR-positive, *Salmonella* PYR-negative).

Some strains of *Yersinia enterocolitica* are incapable of hydrolyzing urea and are biochemically similar to *Shigella* species which may not be differentiated by this algorithm. Most strains (75%) will be urease-positive but may take longer to develop (> 48 hours). In addition to serology, Pyroglutamate aminopeptidase (PYR) activity is effective for differentiating *Yersinia* (PYR-positive) from *Shigella* species (PYR-negative). *Yersinia enterocolitica* colonies will be smaller (< 1mm) than *Shigella*. See EnteroScreen 4™ algorithm (Figure 2).

Strong urease producers, including *Proteus* spp., may rarely produce a false-positive decarboxylase reaction.

Quality Control results are for the EnteroScreen 4™ combination of tests only and not to be confused with the results expected with single biochemical tests.

Gas reaction should be read strictly from the lysine-decarboxylase layer. Bubbles in the urea layer should not be interpretated as gas production.

Only a red color reaction should be considered as positive for Lysine-Deamination (top layer). Usually the negative reaction is characterized by a purple color, but a yellow color may develop due to a strong pH reaction from the middle layer in isolates that are negative for Lysine-Decarboxylase.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as inoculating loops and needles, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents (OxiStrips™, Cat. no. Z93; Indole, DMACA Spot, Cat. no. Z65; PYR Test Kit, Cat. no. Z75), 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:

Note: Refer to "Procedure" section, above, for information concerning inoculation protocol, incubation temperature, and incubation atmosphere.

Test Organisms	Results		
	Urea Agar	Modified Lysine Iron Agar	Lysine Iron Agar

Salmonella enterica ATCC® 14028	Urease-negative (orange color)	Lysine-decarboxylase-positive (purple color) Gas production positive (bubbles in media)	Growth on slant Lysine-deamination-negative (purple slant) H_2S-positive (blackening at base of slant)
Shigella flexneri ATCC® 12022	Urease-negative (orange color)	Lysine-decarboxylase-negative (yellow color) Gas production negative (no bubbles in media)	Growth on slant Lysine-deamination-negative (purple slant) H_2S-negative (purple color at base of slant)
Proteus mirabilis ATCC® 12453	Urease-positive (pink-red color)	Lysine-decarboxylase-negative (yellow color) Gas production variable	Growth on slant Lysine-deamination-positive (red slant) H_2S-positive/negative (purple or slight black color at base of slant)

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

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

EnteroScreen 4™ should consist of four layers within a single tube, which includes three media layers and a hydrophobic layer. The bottom layer should appear opalescent and pale yellow with a slight orange tinge in color. The subsequent layer is a hydrophobic layer, which should appear opaque and white to off-white in color. The next layer and the top slant should appear slightly opalescent and purple in color.

PERFORMANCE CHARACTERISTICS[6,7]

EnteroScreen 4™ was evaluated using 218 clinical isolates. Included among these isolates were 106 isolates *Salmonella* species (23 *S. typhi* and 83 non-*typhi* species of *Salmonella* from 20 serotypes); 56 *Shigella* isolates (representing all four species); and 56 other gram-negative bacteria (representing many lactose-negative *P. aeruginosa* as well as other members of the Enterobacteriaceae family). In this study, Hardy Diagnostics EnteroScreen 4™ correctly identified 100% of all *Salmonella* and *Shigella* isolates. Of the other bacteria, there was a 94.6% agreement between EnteroScreen 4™ and the result obtained from TSI, LIA, and Urea agars. The results of this evaluation demonstrated that Hardy Diagnostics EnteroScreen 4™, when used in conjunction with the algorithm, is an acceptable method for screening of lactose-negative stool isolates that may represent *Salmonella* or *Shigella* species.

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Patent Pending no. 60/333,400

IFU-10410[A]

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

HDQA 2207F [D]