INTENDED USE

REDOX 1 and REDOX 2 (80 ml and EZ Draw 40 ml) media are used in the VersaTREK Automated Microbial Detection (VTI) System for cultivating and recovering microorganisms, especially bacteria and yeasts, from blood and other normally sterile body fluids.

The VersaTREK Connector is used in establishing a sterile monitoring pathway between the VTI instrument and individual bottles being incubated in the instrument.

SUMMARY AND EXPLANATION

The primary objective of culturing blood and other normally sterile body fluids is the growth and detection of very low numbers of microorganisms. The major advantage of an automated or semi-automated blood culture system is the ability to detect the presence of an organism as soon as possible after the growth is initiated. Manual blood culture systems rely on subjective visualization or labor intensive subculturing for detection of positive blood specimens. Blood culture systems, including the VTI System, utilize broth media for the initial step in microbial isolation. Gas consumption (O₂) and/or gas production (CO₂ and other gases, such as N₂ and H₂) are detected by the instrument as the organism grows in the medium. The specially formulated media of the VTI System permit excellent microbial growth and, therefore, effective detection by the instrument sensor. Because the media can be used for all patient populations, specialized formulas, such as those for pediatric and adult, are not required.

PRINCIPALS OF THE PROCEDURE

In the VersaTREK Automated Microbial Detection System, the patient specimen is inoculated into the culture bottles, the VersaTREK Connector is properly positioned, patient information is entered into the system computer, and the bottle is appropriately placed in the VTI instrument for either aerobic or anaerobic incubation. Aerobic bottles are automatically rotated, agitated, or "VorTrexed" during incubation to provide optimal culture conditions. Anaerobic bottles are incubated under stationary conditions.

The VTI instrument detects microbial growth by continuously monitoring gas consumption or production (via the VersaTREK Connector) and reports that growth response.

VersaTREK REDOX 1 and REDOX 2 media may be used in manual methods requiring visual inspection for turbidity, Gram stain, and subculture to plated media for detection of growth.

REAGENTS

VersaTREK REDOX 1 and REDOX 2 80 ml contain 80 ml of medium, permitting use of up to a 10 ml blood specimen. REDOX 1 and REDOX 2 EZ Draw 40 ml contain a nominal medium fill of 40 ml with specific vacuum to draw up to 5 ml of specimen.

REDOX 1, specially formulated as described below, supports recovery of aerobic and facultative microorganisms.

**REDOX 1 FORMULA**

(H₂O) Processed Water 40 ml or 80 ml

(SCP) Soy-Casein Peptone A 2.1% w/v

(NaCl) Sodium Chloride 0.5% w/v

(YE) Yeast Extract 0.1% w/v

(DEX) Dextrose 0.25% w/v

(DVS) Divalent Salts A 0.009% w/v

(SPO) Supplement O 0.33% w/v

(PS) Sodium Polyanetholesulfonate 0.0125% w/v

REDOX 1 is dispensed with added CO₂. Components may be adjusted to meet performance criteria.

REDOX 2, a highly enriched medium found to inactivate aminoglycoside antibiotics, supports recovery of anaerobes, facultative organisms, and some aerobes. Because it is highly reduced to optimize the growth of strict anaerobes, this medium may not support the growth of very strict aerobes.

**REDOX 2 FORMULA**

(H₂O) Processed Water 40 ml or 80 ml

(PPN) Proteose-Peptone N 1.5% w/v

(YE) Yeast Extract 0.5% w/v

(NaCl) Sodium Chloride 0.23% w/v

(DEX) Dextrose 0.5% w/v

(P80) Polysorbate 80 10% 0.075% w/v

(SPAN) Supplement AN 0.8% w/v

(TSC) Trisodium Citrate 0.07% w/v

(SAP) Saponin 0.045% w/v

(HEM) Hemin 0.0005% w/v

(CYS) Cysteine 0.05% w/v

(VK) Vitamin K 0.0001% w/v

(RZN) Resazurin 0.0001% w/v

REDOX 2 is dispensed with added CO₂ and N₂. Components may be adjusted to meet performance criteria.
PRECAUTIONS
1. For In Vitro Diagnostic Use.
2. Refer to Material Safety Data Sheets for complete hazard information
3. WARNING! Potential infectious test specimen. Infectious agents, including HIV and hepatitis B virus, may be present in specimens. Reagents contain material of animal origin and so are potential carriers or transmitters of disease. Follow universal precautions and institutional policy in handling and disposing of infectious agents. (This warning particularly applies to procedures that may cause the creation of aerosols and to the handling of concentrated infectious agents in quantities greater than expected in clinical specimens, both of which should be performed carefully in a biological safety hood.)
4. The VersaTREK Connector contains a sharp recessed needle that is sterile upon first removing the seal, but is potentially contaminated upon termination of use. Follow institutional policy for handling and disposing of blood-contaminated devices. (Do not handle in a "casual" manner. Sterilize prior to disposal. Dispose in an approved sharps container. Do not reuse.)
5. VersaTREK REDOX 1 and REDOX 2 media are specially formulated for production or consumption of gas, therefore pressure may occur within a bottle. The VersaTREK Connector, placed on the bottle immediately following inoculation or upon receipt in the laboratory, automatically vents the bottle through its 0.2 µ filter during incubation in the VT1 instrument and subsequent processing. Bottles used in manual incubation techniques must be vented in a biological safety hood with an approved venting unit or other venting device according to manufacturer instructions.
   • Pressure within the bottle may cause aerosol generation if medium is trapped in the cotton stopper or needle prior to venting. To avoid this, carefully disengage the translucent plastic cover containing the cotton plug from the needle housing and leave in position for a few seconds. If medium has been trapped in the cotton stopper or needle, this will allow the cotton to absorb the liquid, preventing aerosol generation.
   • Do not shake or agitate the bottle prior to venting. Gentle swirling of the bottle is the best way to mix contents.
   • Note: Venting does not affect the anaerobic characteristics of REDOX 2 broth, which are related to the presence of reducing components in the medium, provided the venting does not exceed 15 minutes prior to entry into the VT1 instrument.
   • Do not tip, shake or invert a bottle until it has vented completely and the VersaTREK connector or vent has been removed.
6. Use only needle-locking syringes or one-piece needle-syringe units.
7. Visually inspect all bottles for contamination, cracks, or other signs of deterioration. Do not use bottles that appear turbid or damaged.
8. If seal is broken, DO NOT USE VersaTREK Connector and discard in sharps container.

STORAGE
Store REDOX 1 and REDOX 2 media at 15-30°C. Protect from light. Store VersaTREK Connectors at 15-30°C.

SPECIMEN COLLECTION PRINCIPLES
The timing of the blood sampling is critical for optimal recovery of pathogenic microorganisms.1,8
Recommended procedure is to obtain two culture sets, each from a different body site.6,8
Due to sporadic organism distribution in the blood, larger sample volumes (up to 30 ml) have been shown to significantly increase the likelihood of detection.1,2,5-10 Clinical data for the VTI System supports the use of adult specimens as small as 1-5 ml and pediatric specimens of 0.1-3 ml. Note that a minimum of 0.5 ml of blood is recommended for recovery of Haemophilus influenzae and Neisseria species.
REDOX 1 and REDOX 2 80 ml bottles accommodate specimens up to 10 ml, REDOX 1 and REDOX 2 EZ Draw 40 ml bottles up to 5 ml.

PROCEDURE
Materials Provided:
VersaTREK REDOX 1 EZ Draw 40 ml with Stir Bar
VersaTREK REDOX 2 EZ Draw 40 ml
VersaTREK REDOX 1 80 ml with Stir Bar
VersaTREK REDOX 2 80 ml
VersaTREK Connectors

Materials Required but not Provided:
2% tincture of iodine or 10% povidone solution
Alcohol swabs
Approved venting unit
Autoclave
Bandages
Plated culture media for subculture
Sterile syringes and needles, Blood Collector or multi-draw adapter
Surgical or other suitable tape
Tourniquet
VersaTREK Automated Microbial Detection System

SPECIMEN COLLECTION
Obtain specimens according to the techniques and procedures established by institutional policy. Guard against contamination of the specimen during collection and processing.
If blood is to be drawn for other procedures in addition to culture, obtain the specimen for culture first to minimize the potential for contamination of the culture.
1. Equilibrate the bottle of medium to room temperature. Label with patient information.
2. Disinfect the top of the bottle stopper. DO NOT REMOVE THE ALUMINUM SEAL OR METAL SCREW CAP.
3. Set up the specimen collection device according to institutional policy and, if applicable, manufacturer instructions.

SYRINGE AND NEEDLE: Assemble the sterile needle and syringe or use a sterile needle-syringe combination. Loosen but do not remove the needle shield.

BLOOD COLLECTOR: Remove the Blood Collector from the package. DO NOT CLAMP THE TUBING. Follow the manufacturer's directions for use of the specific device. Loosen but do not remove the needle shield at the venipuncture collection end of the tubing.
DIRECT DRAW ADAPTOR (REDOX 1 and REDOX 2 EZ Draw 40 ml bottle only): Assemble the direct draw adaptor according to the manufacturer's directions. Insert the REDOX 1 and REDOX 2 EZ Draw 40 ml bottle onto the holder and needle until the leading edge of the stopper meets the guide line on the holder. The bottle will retract slightly. Leave in this position.

4. Select and prepare the venipuncture site. Apply the tourniquet. Palpate the area and select the site(s). Cleanse with sterile 70% isopropyl alcohol. Apply 1-2% tincture of iodine or 10% povidone iodine. Allow to dry.

NOTE: For patients with known hypersensitivity to iodine, a double application of sterile 70% alcohol is recommended. Do not palpate the prepared area after cleansing.

5. Obtain the specimen according to institutional policy and the instructions of the device manufacturer. REDOX 1 and REDOX 2 80 ml bottles permit a 10 ml fill; REDOX 1 and REDOX 2 EZ Draw 40 ml bottles permit a 5 ml fill.

SYRINGE AND NEEDLE: Remove the needle shield and perform venipuncture, withdrawing up to 5 or 10 ml of blood per bottle to be cultured. Add this blood to the culture medium in the bottle by puncturing the bottle stopper with the needle and syringe.

BLOOD COLLECTOR: Remove the needle shield and perform venipuncture, allowing blood to reach the end of the stopper-puncturing needle. Puncture the bottle stopper with the needle. LOOSEN THE TOURNIQUET AS SOON AS BLOOD STARTS TO FLOW. Fill the bottle to the designated volume guides on the bottle label.

The REDOX 1 and REDOX 2 80 ml bottle label displays 5 ml sample fill increments. When using a closed collection system (blood collector), observe the fill procedure carefully and remove the stopper-perforating needle when the desired sample (up to 10 ml) is achieved.

The REDOX 1 and REDOX 2 EZ Draw 40 ml bottle contains a vacuum to draw up to a 5 ml sample. It is normal for the bottle not to fill completely.

When blood flow into the bottle slows, remove the stopper-puncturing needle. After removing the needle from the stopper (the blood flow ceases due to the needle slide valve), fill subsequent bottles as required.

DIRECT DRAW ADAPTOR (REDOX 1 and REDOX 2 EZ Draw 40 ml bottle only): Lower the patient's arm until it is in a vertical position. Position the REDOX 1 or REDOX 2 EZ Draw bottle vertically with the stopper uppermost prior to penetration. The medium should not be in contact with the bottle stopper during venipuncture.

Push the bottle onto the bottom of the holder, puncturing the bottle stopper. If the needle is in the vein, blood will flow into the bottle. If blood does not flow, remove the bottle at once and repeat the procedure with a new culture bottle.

When blood flow into the bottle slows, remove the bottle from the holder. It is normal for the bottle not to be completely filled. Loosen the tourniquet when the final bottle to be drawn is almost full.

6. Discard the collection device according to institutional policy.
7. Bandage the patient's arm.
8. Disinfect the top of the bottle stopper.
9. Mix the blood and broth by inverting 4-5 times.

NON-BLOOD SPECIMEN PREPARATION
Prior to incubation, add sterile defibrinated sheep blood or other supplements (Supplement B or sterile horse blood) to bottles containing non-blood specimens (normally sterile body fluids) to support growth of fastidious organisms such as *Haemophilus influenzae* and *Neisseria gonorrhoeae* which may be present in the specimen.

PREINCUBATION
Preincubation of bottles is not recommended. However, if preincubated bottles are received, they should be checked for indications of microbial growth based on turbidity and, if appropriate, Gram stain. (SEE PRECAUTIONS.)

If the Gram stain confirms growth, the bottle should not be further incubated but should proceed directly to identification and susceptibility testing per institutional protocol.

If growth cannot be confirmed, equilibrate (cool) the medium to ambient temperature (24 ± 4°C), approximately 40-60 minutes. Proceed with steps below.

INCUBATION
1. Invert the bottle gently 4-5 times to mix.
2. Again, disinfect the top of the bottle stopper.
3. Aseptically remove the VersaTREK Connector seal, being careful not to contaminate the recessed needle. Position the VersaTREK Connector on the top of the disinfected bottle, press vertically down to puncture the bottle stopper, and completely seat the VersaTREK Connector. Proper positioning of the VersaTREK Connector will assure proper connection of the bottle to the VTI instrument.

NOTE: Do not mix bottle contents while the VersaTREK Connector is on the bottle.
4. Record the desired patient information in the VTI computer.
5. Place the bottle into the designated VTI instrument location.
6. When the VTI instrument indicates via a steady red light that a particular bottle location contains a positive culture, remove the bottle according to the Procedures specified in the VTI User Manual. Do not tip or invert the bottle while removing it from the instrument.

Note: If the bottle is tipped or inverted during removal, allowing fluid into the VersaTREK Connector needle, it may be necessary to vent the bottle by replacing the VersaTREK Connector or by using an alternate method (approved venting unit). If an alternative method is used, venting should occur in a biological safety hood.

7. Allow the bottle to vent through the VersaTREK Connector, about 3 seconds in the case of a positive culture. Remove the VersaTREK Connector from the bottle and disinfect the bottle stopper before proceeding to POST-INCUBATION PROCEDURES. (See PRECAUTIONS.)

INCUBATION BY MANUAL TECHNIQUES
Follow institution's procedures for processing manual blood culture bottles.

POST-INCUBATION PROCEDURES
1. Obtain specimens for Gram stain and subculture using an approved venting unit, a syringe and needle, or other device approved by institutional policy. (See PRECAUTIONS.)
2. Sterilize bottle contents prior to disposal.
USER QUALITY CONTROL

A Certificate of Analysis is included with each lot of REDOX culture medium. Each lot conforms with TREK's quality assurance criteria and also with CLSI specifications as stated in Quality Assurance for Commercially Prepared Microbiological Culture Media.

QC CULTURAL RESPONSE

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC</th>
<th>REDOX 1</th>
<th>REDOX 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>25923</td>
<td>growth</td>
<td>--</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>6305</td>
<td>growth</td>
<td>growth</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>27853</td>
<td>growth</td>
<td>--</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>25285</td>
<td>--</td>
<td>growth</td>
</tr>
</tbody>
</table>

a. Growth within 96 hours.
b. 1.0 ml challenge dose containing no greater than 300 CFU.

LIMITATIONS

1. A Gram stained smear from culture medium may contain small numbers of non-viable but stainable bacteria from media constituents, staining reagents and devices.
2. It is difficult to avoid an occasional contaminant in a blood culture. The situation is further complicated by the fact that some common contaminants (i.e., Staphylococcus epidermis, Propionibacterium acnes) have been reported as etiological agents of endocarditis and sepsis. Finding the organism repetitively in multiple blood sets from a patient is the best evidence that the organism is not a contaminant.
3. Prior to incubation in the VTI Instrument, equilibrate (cool) preincubated bottles to ambient temperature. An uncooled, preincubated bottle (>28°C) may cause the VTI Instrument to produce an error.
4. REDOX 1 and REDOX 2, when used to culture non-blood specimens (normally sterile body fluids), may require added blood or other supplements to support growth, particularly of fastidious organisms such as Haemophilus influenzae and Neisseria gonorrhoeae.
5. It is possible to have a septicaemia caused by an organism that will not grow, or grow and not be detected in the VTI. If such an organism is suspected, additional, alternative methods for recovery or detection should be considered. VersaTREK Myco medium is recommended for cultivating and detecting Mycobacterium species. The VTI System is not recommended for cultivating viruses.
6. To accommodate the metabolic needs of a broad range of organisms, optimal recovery is achieved when both media are inoculated as a set with each patient draw.
7. Although some aerobes have been recovered from anaerobic broth, strict aerobes may not be detected because of the highly reduced nature of the medium.
8. "Neutralization" of antimicrobial activity by dilution in culture media varies depending on the dosage level, susceptibility of the microorganisms, and timing of specimen collection. Use of supplemental additives should be considered in appropriate situations, for example, the addition of beta-lactam inactivating enzymes when beta-lactam therapy is being employed.
9. Efficient recovery of Haemophilus influenzae and Neisseria species requires a minimum inoculum of 0.5 ml blood.
10. Efficient recovery of some strict aerobes in the REDOX 1 EZ Draw 40 ml bottle format depends on proper venting to establish sufficient oxygen in the headspace.
11. Overfilling the bottle may cause a false positive result.

PERFORMANCE CHARACTERISTICS

Data from in-house and clinical studies of the VTI System demonstrate good recovery and detection of common as well as rare microorganisms normally isolated from blood and other body fluids.

### TABLE 1.

<table>
<thead>
<tr>
<th>Organisms detected, VTIa Classification/Genus</th>
<th>No./Different Spp. Detectedb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic and facultative gram-positive cocci:</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>6</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>17</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
</tr>
<tr>
<td>Aerobic and facultative gram-negative rods:</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>30</td>
</tr>
<tr>
<td>Other</td>
<td>35</td>
</tr>
<tr>
<td>Aerobic gram-negative cocci:</td>
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</tr>
<tr>
<td>Neisseria sp.</td>
<td>4</td>
</tr>
<tr>
<td>Aerobic and facultative gram-positive rods:</td>
<td></td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>3</td>
</tr>
<tr>
<td>Listeria sp.</td>
<td>1</td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td>3</td>
</tr>
<tr>
<td>Erysipelothrix sp.</td>
<td>1</td>
</tr>
<tr>
<td>Yeasts:</td>
<td></td>
</tr>
<tr>
<td>Candida sp.</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>3</td>
</tr>
<tr>
<td>Anaerobic gram-positive rods:</td>
<td></td>
</tr>
<tr>
<td>Clostridium sp.</td>
<td>7</td>
</tr>
<tr>
<td>Lactobacillus sp.</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
<tr>
<td>Anaerobic gram-positive cocci:</td>
<td></td>
</tr>
<tr>
<td>Peptostreptococcus sp.</td>
<td>6</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
</tr>
<tr>
<td>Anaerobic gram-negative rods:</td>
<td></td>
</tr>
<tr>
<td>Bacteroides sp.</td>
<td>6</td>
</tr>
<tr>
<td>Fusobacterium sp.</td>
<td>3</td>
</tr>
<tr>
<td>Porphyromonas sp.</td>
<td>1</td>
</tr>
</tbody>
</table>

a. ATCC strains (<500 organisms/bottles tested with 5 ml fresh human blood) and isolates from clinical blood specimens.
b. In-house and clinical studies detected >1400 different organisms.

d. A false negative is defined as a specimen that has a positive subculture but was not flagged clinically significant.
d. A false positive is defined as a specimen that flagged positive by the VTI instrument but has a negative subculture.
d. A false negative is defined as a specimen that has a positive subculture but was not flagged positive by the instrument.

d. C. Statistically significant differences were due to better recovery of gram-positive organisms, coagulase-negative organisms and Streptococcus pneumoniae by the VTI instrument.

d. A false positive is defined as a specimen that flagged positive by the VTI instrument but has a negative subculture.

At seven clinical sites, equal volumes of blood specimens 0.5-5 ml adult, 0.1-3 ml pediatric (newborn to 14 years) were collected, inoculated, and incubated in the VTI System and another system. Thirty-nine body fluids determined to be positive by manual methods were also evaluated in the VTI Instrument; all yielded positive test results.

### TABLE 2.

| Clinical Resultsa summary % (actual number(s)) detected byb TOTAL VersaTREK BACTEC |
|-----------------------------|-----------------|----------------|----------------|
| **AEROBIC CULTURES**        |                 |                |                |
| Adult                       | 6442            | 6442           |                |
| Pediatric                   | 1432            | 1432           |                |
| Positives                   | 616             | 80 (493/616)   | 69 (425/616)   |
| Adult                       | 497             | 79 (392/497)   | 71 (353/497)   |
| Pediatric                   | 119             | 85 (101/119)   | 60 (72/119)    |
| Clinically Significant      | 434             | 86 (373/434)   | 78 (338/434)   |
| Adult                       | 363             | 84 (305/363)   | 78 (282/363)   |
| Pediatric                   | 71              | 94 (67/71)     | 79 (56/71)     |
| False Positivec             |                | 0.9            | NA             |
| False Negativc              |                | 0.1            | 0.1            |
| Contaminatisd               |                | 1.5            | 1.1            |
| **ANAEROBIC CULTURES**      | 6442            | 6442           |                |
| Adult                       | 6442            | 6442           |                |
| Pediatric                   | 0               | 0              |                |
| Positives                   | 388             | 82 (318/388)   | 66 (256/388)   |
| Clinically Significant      | 298             | 86 (256/298)   | 75 (224/298)   |
| False Positive              |                | 1.0            | NA             |
| False Negative              |                | 0.2            | 0.2            |
| Contaminatisd               |                | 1.0            | 0.5            |
| **Clinically Significant**  |                 |                |                |
| Episodes                    | 350             | 87 (305/350)   | 76 (273/350)   |
| Adult                       | 292             | 86 (252/292)   | 75 (228/292)   |
| Pediatric                   | 58              | 91 (53/58)     | 76 (45/58)     |

a. Does not include body fluids other than blood.
b. Statistically significant differences were due to better recovery of gram-positive organisms, coagulase-negative organisms and Streptococcus pneumoniae by the VTI instrument.
c. A false positive is defined as a specimen that flagged positive by the VTI instrument but has a negative subculture.
d. A false negative is defined as a specimen that has a positive subculture but was not flagged positive by the instrument.
REFERENCES:

Symbol Legend

<table>
<thead>
<tr>
<th>REF</th>
<th>Catalogue number</th>
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</thead>
<tbody>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
</tr>
<tr>
<td></td>
<td>Manufacturer</td>
</tr>
<tr>
<td></td>
<td>Temperature limitation (storage temp.)</td>
</tr>
<tr>
<td></td>
<td>Use by (expiration date)</td>
</tr>
<tr>
<td>LOT</td>
<td>Lot number</td>
</tr>
<tr>
<td></td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>EC REP</td>
<td>Authorized European Representative</td>
</tr>
<tr>
<td></td>
<td>Contains sufficient for &lt;n&gt; tests</td>
</tr>
<tr>
<td></td>
<td>Keep away from heat</td>
</tr>
</tbody>
</table>

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Lenexa, KS 66215, USA
www.remel.com
1-800-255-6730

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For technical information contact your local distributor.

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