ASSAY PRINCIPLE

The SMART-SPOT® device consists of a membrane mounted to a hard carrier. The membrane is the reactive unit and should not be touched. The membrane has 7 invisible test “spots”. Each of the first 6 spots is a specific antigen, as indicated in the diagram below, and the 7th spot is a Test Control. The Test Control spot insures that a specimen was added and that all of the reagents worked properly.

During the first incubation with the diluted specimen, any antibody that is reactive with the specific antigen will bind to the spot. After washing to remove the rest of the sample, Enzyme Conjugate-1 is added. After another series of washes, Enzyme Conjugate-2 is added. If antibodies have been bound to the spots, the Enzyme Conjugates will then bind to these antibodies. After another series of washes, a Chromogen is added. If the Enzyme Conjugates have bound to a spot, the Chromogen will turn the spot from invisible to a shade of purple. The strip is rinsed to stop the reaction and any purple color in an antigen spot is indicative of antibody present to that specific antigen. The Test Control spot MUST be positive (a shade of purple) in order for the test to be valid.

The SMART-SPT6EM assay tests for the following:

Position 1: CPIL (Clostridium piliforme)
Position 2: ECT (Ectromelia Virus)
Position 3: LCM (Lymphocytic Choriomeningitis Virus)
Position 4: HANT (Hantaan Virus)
Position 5: MAd (Murine Adenovirus Types 1 + 2)
Position 6: MCMV (Murine Cytomegalovirus)
Position 7: Test Control
REAGENTS PROVIDED

- **Test Strips**: Eight (8) SMART-SP®T devices
- **Enzyme Conjugate -1**: One (1) 20ml bottle of anti-mouse IgG conjugate-1
- **Enzyme Conjugate -2**: One (1) 20ml bottle of anti-mouse IgG conjugate-2
- **Chromogen**: One (1) 20ml bottle of substrate
- **Blocking Buffer**: Three (3) Bottles of powdered blocking buffer
- **Specimen Diluent/Wash Buffer Concentrate**: Three (3) 25ml bottles of concentrated (20X) buffer
- **Incubation Tray**: One (1) 8-channel incubation tray
- **Test Tubes**: Eight (8) Specimen dilution tubes
- **Transfer Pipettes**: Eight (8) Diluted specimen transfer pipettes

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettes capable of delivering 20µl and 2ml
- Test tube rack to hold the diluted specimens
- Squeeze bottle for washing strips
- Distilled or reagent grade water

REAGENT STORAGE

Store the kit reagents and SMART-SP®T devices strips between 2 - 8º C.
Store the diluted Specimen Diluent/Wash Buffer between 2 - 8º C. This diluted buffer is good for up to 10 days. Do not add fresh buffer to old buffer.

SERUM COLLECTION AND HANDLING

This test utilizes the specimen’s serum: coagulate the blood and remove the serum. The use of “bloody” sera is contraindicated. Serum samples should be refrigerated as soon as possible after collection and tested within 48 hours. If the specimen is not to be tested within 48 hours after collection, the serum sample should be stored at 0ºC or lower.
Do not heat-inactivate serum and avoid repeated freezing and thawing of samples.
Vortex (mix well) all samples before using.
**Do not use pooled specimens as this will adversely affect the performance of the assay.**

Test samples are diluted 1:101 in the Specimen Diluent/Wash Buffer (20µl of sera + 2ml of Specimen Diluent/Wash Buffer)
**All Reagents must be at room temperature before beginning the assay.**

PROCEDURAL NOTES

Allow all reagents and samples to come to room temperature before testing. It is normal for the concentrated Diluent/Wash Buffer to crystallize when cold. The crystals will re-dissolve once the solution returns to room temperature.
Do not use reagents beyond the expiration date printed on the label.
Do not reuse provided test tubes, transfer pipettes, or channels in the incubation tray or the SMART-SP®T device.
Do not touch the membrane portion of the SMART-SP®T device
Do not inter-mix conjugates between different kits or different lot numbers of the same kit: these components are balanced to work together as a unit. The chromogen, blocking buffer, and Specimen Diluent/Wash Buffer are universal reagents and can be inter-changed between all SMART-SP®T kits.

The SMART-SP®T devices are shipped flat and must be “bent” into the working configuration at the time of use. Place the device on a dry flat surface and gently bend the carrier up so that the mouse is on the top left side. The membrane will sit flat against the incubation tray floor and the carrier will be straight up in the incubation channel to hold the device in place.
PROCEDURE
See attached procedure guide. All procedures and reagents are at room temperature (15 – 25°C).

TROUBLESHOOTING
If the Test Control spot is not visible the test is not valid. Recheck your procedure and insure that all reagents are at room temperature before starting the assay.
If all the spots have visible color or there is a high degree of background staining usually indicates incomplete washing. Washing is extremely important in all assays, and incomplete washing leaves behind excess reagents that may give false positive results. Also check the three “T”s": Time, Temperature and Technique. Time: insure that the timing on the incubation stages is adhered to. Temperature: temperatures above 25°C may adversely affect the assay; Technique: check all pipettes to insure that they are properly delivering the correct volume to produce a 1:101 dilution of the specimens.

READING RESULTS
Allow the test membrane to dry for at least 30 minutes before reading and should be read within 48 hours after the start of the drying period. Drying time is dependent upon lab conditions (temperature and humidity). The Test Control spot must be visible for the test to be valid. Any purple color in a specific antigen spot is indicative of antibody present to that specific antigen and further testing is required by an alternate method.

EXPECTED VALUES
The normal value is Negative. Studies have shown that antibodies may take up to 21 days to appear after exposure; therefore, negative specimen results should be reviewed in relation to a possible exposure date. SMART-SPOT is designed as a screening assay and positive specimen results should be confirmed by an alternate method. Although uncommon, false positive results may occur from non-specific antibodies binding to the media in which the antigen is derived.

This product is warranted to perform as described in the labeling provided that: the product is stored and used as directed; used before the expiration dating; and adequate quality control is performed. No other warranty is implied, nor are we liable for any consequential damages arising out of the aforesaid warranty.

Biotech Trading Partners, Inc
1042B N El Camino Real, Suite 341
Encinitas, CA  92024
Phone:   1-760-578-6176
Fax:   1-267-295-8218
Email:    info@biotechtradingpartners.com
Web Site:  www.biotechtradingpartners.com

A graduate of the SMART program
SMART-SPOT®
Sentinel Panel Test
SMART-SPOT® PROCEDURE GUIDE
6-Analyte Expanded Panel (Mouse Sera), Catalog #SMART-SPT6EM

INITIAL SETUP
1. Make the working dilution of the Specimen Diluent/Wash Buffer by bringing one bottle of the 20X Specimen Diluent/Wash Buffer Concentrate to 500ml with distilled water. Add the entire contents of one bottle of the Blocking Buffer. **MIX WELL**
2. Prepare 1:101 dilution of the specimens as follows:
   - Add 2ml of diluted Specimen Diluent/Wash Buffer made in step one to a provided test tube
   - Add 20µl of each specimen to the appropriate tube. **MIX WELL**

SERUM INCUBATION STAGE
3. Without touching the membrane, remove the required number of SMART-SPOT devices and reseal the pouch. (Unused devices must be kept sealed in a dry environment) The working configuration of the device is that the membrane is at a 90° angle to the carrier. The membrane sits flat on the floor of the incubation channel and the carrier sits up straight in the channel and holds the device in place. Using a sharpie, label the carrier portion with your specimen ID number.
4. Insert the SMART-SPOT device into the appropriate channel in the incubation tray insuring that the membrane is against the floor of the channel and that the carrier is securely wedged into the channel.
5. Using the provided transfer pipettes, transfer the all of the diluted specimen from step 2 into the appropriate channel in the incubation tray. Rock the tray several times to mix and insure that the membrane is fully covered by the diluted specimen.
6. Incubate at room temperature for **30 MINUTES**.

WASH STAGE
7. After 30 minutes, pick up the tray and rest it against the palm of your hand with your thumb gently crossing over the top of the device carriers. Invert the tray and dump (the carrier will hold the device in place). Wash the strip by directing the stream of the working dilution of the Specimen Diluent/Wash Buffer directly along the strip and completely fill each channel to the top. Dump again.
8. Repeat the above step four more times for a total of **5 WASHES**.
9. After the last wash, shake the tray 5-6 times to remove excess liquid.

CONJUGATE-1 INCUBATION STAGE
10. Add 2ml of CONJUGATE-1 to each channel with a device and rock the tray several times to mix and insure that the membrane is fully covered.
11. Incubate at room temperature for **20 MINUTES**.

WASH STAGE
12. After 20 minutes, pick up the tray and rest it against the palm of your hand with your thumb gently crossing over the top of the device carriers. Invert the tray and dump (the carrier will hold the device in place). Wash the strip by directing the stream of the working dilution of the Specimen Diluent/Wash Buffer directly along the strip and completely fill each channel to the top. Dump again.
13. Repeat the above step four more times for a total of **5 WASHES**.
14. After the last wash, shake the tray 5-6 times to remove excess liquid.

CONJUGATE-2 INCUBATION STAGE
15. Add 2ml of CONJUGATE-2 to each channel with a device and rock the tray several times to mix and insure that the membrane is fully covered.
16. Incubate at room temperature for **10 MINUTES**.

WASH STAGE
17. After 10 minutes, pick up the tray and rest it against the palm of your hand with your thumb gently crossing over the top of the device carriers. Invert the tray and dump (the carrier will hold the device in place). Wash the strip by directing the stream of the working dilution of the Specimen Diluent/Wash Buffer directly along the strip and completely fill each channel to the top. Dump again.
18. Repeat the above step four more times for a total of **5 WASHES**.
19. After the last wash, shake the tray 5-6 times to remove excess liquid.

CHROMOGEN STAGE
20. Add 2ml of CHROMOGEN to each channel with a device and rock the tray several times to mix and insure that the membrane is fully covered.
21. Incubate at room temperature for **5 MINUTES**.

STOP STAGE / DRYING STAGE
22. After 5 minutes, pick up the tray and rest it against the palm of your hand with your thumb gently crossing over the top of the device carriers. Invert the tray and dump (the carrier will hold the device in place). Wash the strip by directing the stream of the working dilution of the Specimen Diluent/Wash Buffer directly along the strip and completely fill each channel to the top. Repeat one more time for a total of 2 washes.
23. Remove the device from the tray and place on a paper towel to dry for **at least** 30 minutes. The strips should read within 48 hours after the start of the drying period. Any purple color present on the specific spot is indicative of antibody present to that antigen and further testing is suggested.
Multi-Species EIA Serology Assays
BTP-96200  QnE Hyaluronic Acid (HA) Quantitative ELISA assay

Mouse ELISA Serology Assays
SMART-M10  MVM, Minute Virus of Mice
SMART-M11  Sendai Virus
SMART-M12  PVM, Pneumonia Virus of Mice
SMART-M13  REO-3, REO Virus Type 3
SMART-M14  TMEV (GDVII), Theiler’s Murine Encephalomyelitis Virus
SMART-M15  LCM, Lymphocytic Choriomeningitis Virus
SMART-M16  Ectromelia Virus
SMART-M17  MHV, Mouse Hepatitis Virus
SMART-M18  Polyoma Virus
SMART-M19  EDIM, Epizootic Diarrhea of Infant Mice, (Mouse Rotavirus)
SMART-M23  K, Mouse Pneumonitis Virus
SMART-M24  MCMV, Mouse Cytomegalovirus
SMART-M25  MAD 1, Murine Adenovirus FL
SMART-M26  MAD 2, Murine Adenovirus K87
SMART-M27  MPUL, Mycoplasma pulmonis
SMART-M28  MPV (rVP2), Mouse Parvovirus
SMART-M30  CARB, Cilia-Associated Respiratory Bacillus
SMART-M31  ECUN, Encephalitozoon cuniculi
SMART-M32  CPIL, Tyzzer’s Disease (Clostridium piliforme)
SMART-M33  Hantaan Virus
SMART-M35  Murine Norovirus

Rat ELISA Serology Assays
SMART-R11  Sendai Virus
SMART-R12  PVM, Pneumonia Virus of Mice
SMART-R13  REO-3, REO Virus Type 3
SMART-R14  TMEV (GDVII), Theiler’s Murine Encephalomyelitis Virus
SMART-R15  LCM, Lymphocytic Choriomeningitis Virus
SMART-R20  SDAV/RCV, Rat Coronavirus/Sialodacroadenitis Virus
SMART-R21  KRV, Kilham Rat Virus
SMART-R22  H1, Toolan’s Virus
SMART-R25  MAD-1,2, Murine Adenovirus FL/K87
SMART-R27  MPUL, Mycoplasma pulmonis
SMART-R29  RPV (rVP2) Rat Parvovirus
SMART-R30  CARB, Cilia-Associated Respiratory Bacillus
SMART-R31  ECUN, Encephalitozoon cuniculi
SMART-R32  CPIL, Tyzzer’s Disease (Clostridium piliforme)
SMART-R33  Hantaan Virus

Non-Human Primate ELISA Serology Assays
4810  Herpes B-Virus
4811  Simian Immunodeficiency Virus (SIV)
4812  Simian Retrovirus (SRV)
4813  Simian T-Lymphotropic Virus (STLV)
4814  Simian Measles Virus

Multiple Analyte SPOT Serology Assays
MOUSE:  SMART-SPT9M
Simultaneous & discrete detection of antibodies to EDIM; Mouse Hepatitis Virus; Mycoplasma pulmonis; Mouse Parvovirus, Minute Virus of Mouse; Pneumonia Virus of Mice; REO-3; Sendai, and TMEV (GD7) in mouse serum

MOUSE:  SMART-SPT6EM
Simultaneous & discrete detection of antibodies to Clostridium piliforme; Ectromelia Virus; LCM; Hantaan Virus; Mouse Adenovirus FL/K87; Mouse Cytomegalovirus in mouse serum

RAT:  SMART-SPT8R
Simultaneous & discrete detection of antibodies to KRV; Mycoplasma pulmonis; Pneumonia Virus of Mice; SDAV/RCV; REO-3; Rat Parvovirus; Sendai, and TMEV (GD7) in rat serum.

NON-HUMAN PRIMATE  SIMIAN-SPT5S
Simultaneous & discrete detection of antibodies to Herpes B-Virus; SIV; STLV; SRV; and Measles in non-human primate serum.