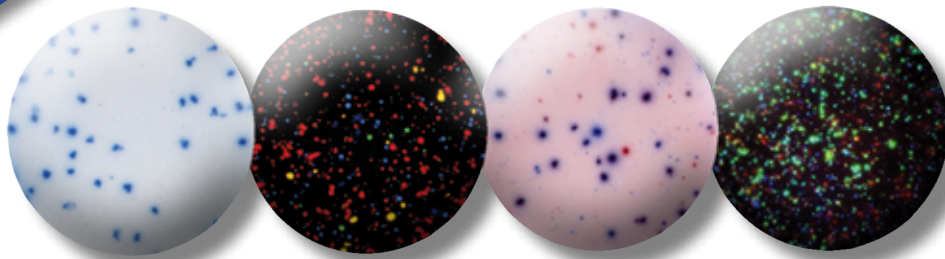# ImmunoSpot®

The ELISPOT Source

• PRESENTS •

IMMUNE MONITORING:
Its Horizons in Theory and Practice

FIVE-DAY WORKSHOP

A Hands-On Training Course
on the use of
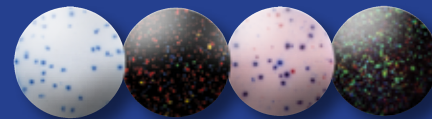## ELISPOT Assays for
Antigen-Specific Immune Monitoring

HANDS-ON ELISPOT TRAINING
5-DAY WORKSHOP

DAY 1: MONDAY

8:30 A.M.

Meet in Hotel Lobby for Transportation to CTL

9:00 A.M. – 9:45 A.M.

Welcome, Tour of Building
presented by Prof. Dr. Paul V. Lehmann

9:45 A.M. – 11:00 A.M.   LECTURE

The unique features of ELISPOT for immune monitoring:
General and T cells
presented by Prof. Dr. Paul V. Lehmann

11:00 A.M. – 11:15 A.M   COFFEE BREAK

11:15 A.M. – 11:45 A.M.   LECTURE

Analyzing T cell ELISPOT assay results I —
SmartCount™ and AutoGate™: Statistics-based automated
size gating of T cell ELISPOTs permits obtaining objective,
user-independent spot counts
presented by Dr. Alexey Karulin

11:45 A.M. – 12:30 P.M.   LIVE DEMO / TUTORIAL

Analysis of single-color enzymatic T cell assays —
Basic features of ImmunoSpot®
presented by Tameem Ansari
- Scanning, single-color visible light
- Selecting spot category
- Automatic background corrections, SmartWell™
- Areal background corrections: Background balance, edge filter
- AutoGate™

12:30 P.M. – 1:15 P.M   LUNCH BREAK

1:15 P.M. – 1:45 P.M.   HANDS-ON TRAINING / Q&A

Analysis of single-color enzymatic T cell assays —
Basic features of ImmunoSpot®
presented by Tameem Ansari, Jodi Hanson, Richard Caspell &
Villian Naeem
- Scanning, single-color visible light
- Selecting spot category
- Automatic background corrections, SmartWell™
- Areal background corrections: Background balance, edge filter
- AutoGate™
- QC — Verify gate
- Multi-parameter AutoCount™

1:45 P.M. – 2:15 P.M.   LIVE DEMO / TUTORIAL

Analysis of single-color enzymatic T cell assays —
Advanced features of ImmunoSpot®
presented by Tameem Ansari
- Manual gating
- Audit trails
- Multi-parameter AutoCount™
- QC — Audit trails
- View/Print/Export
- SpotMap™

2:15 P.M. – 2:45 P.M.   HANDS-ON TRAINING / Q&A

Analysis of single-color enzymatic T cell assays —
Advanced features of ImmunoSpot®
presented by Tameem Ansari, Jodi Hanson, Richard Caspell &
Villian Naeem
- Manual gating
- Audit trails
- Multi-parameter AutoCount™
- QC — Audit trails
- View/Print/Export
- SpotMap™
- Soliciting CTL Support: Annotation tool

2:45 P.M. – 3:15 P.M.   LECTURE

Double-color enzymatic T cell ELISPOT
presented by Prof. Dr. Paul V. Lehmann

3:15 P.M. – 3:45 P.M   COFFEE BREAK

3:45 P.M. – 4:30 P.M.   LIVE DEMO / Q&A

Double-color enzymatic T cell ELISPOT analysis
presented by Jodi Hanson

6:00 P.M.   TOUR OF AMISH COUNTRY & DINNER

DAY 2: TUESDAY

9:00 A.M.

Meet in the Hotel Lobby for Transportation to CTL
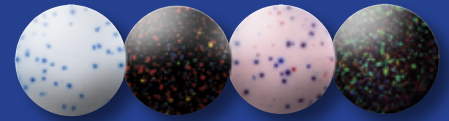
9:30 A.M. – 10:30 A.M.   LIVE DEMO

Performance of a three-color T cell ELISPOT assay:
Thawing of PBMC, three-color counting of live/dead/apoptotic
cells, plating PBMC
presented by Jodi Hanson

Demonstrating:
- Prewetting of plate
- Detection of IFN-γ, Granzyme B, and TNF-α in 3-color
  ImmunoSpot® Assay

HANDS-ON ELISPOT TRAINING
5-DAY WORKSHOP

10:30 A.M. – 11:00 A.M.   LIVE DEMO / TUTORIAL / HANDS-ON

Demo and practice of L/D/A scanning and analysis
presented by Dr. Srividya Sundararaman

11:00 A.M. – 11:15 A.M   COFFEE BREAK

11:30 P.M. – 12:00 NOON   LECTURE

Freezing-thawing of primary cells (PBMC, splenocytes, etc.)
presented by Prof. Dr. Paul V. Lehmann

12:00 NOON – 12:45 P.M.   LUNCH BREAK

12:45 P.M. – 1:15 P.M.   LECTURE

The (lack of) influence of apoptotic cells on T cell function — pre- and post-LDA counting for PBMC functionality
presented by Dr. Srividya Sundararaman

1:15 P.M. – 1:30 P.M.   LECTURE

Cryopreserved PBMC: Why overnight resting is generally not advisable
presented by Richard Caspell

1:30 P.M. – 2:00 P.M.   LECTURE

The theory of multicolor ELISPOT analysis — unambiguously identifying cells that do or do not co-express analytes
presented by Dr. Alexey Karulin

2:00 P.M. – 2:45 P.M.   LIVE DEMO / TUTORIAL

Four-color fluorescent ELISPOT scanning and analysis
presented by Richard Caspell & Jodi Hanson
- Four-color scanning
- No cross-bleeding of CTL fluorochromes
- Four-color analysis of B cell assay (no coexpression possible)
- Three-color analysis of T cell assay (coexpression expected)

2:45 P.M. – 3:00 P.M.   COFFEE BREAK

3:00 P.M. – 3:45 P.M.   HANDS-ON TRAINING / Q&A

Four-color fluorescent ELISPOT scanning and analysis
presented by Richard Caspell, Jodi Hanson, Tameem Ansari & Villian Naeem
- Four-color scanning
- No cross-bleeding of CTL fluorochromes
- Four-color analysis of B cell assay (no coexpression possible)
- Three-color analysis of T cell assay (coexpression expected)
- Linearity for verification of coexpression

3:45 P.M. – 4:30 P.M.   LECTURE

Cut-offs for positive ELISPOT results: Statistics and how to experimentally increase the chance for the reliable detection of rare cells
presented by Dr. Alexey Karulin

4:30 P.M. – 5:00 P.M.   LECTURE

ELISPOT assays in 384-well format
presented by Jodi Hanson

6:00 P.M.   TOUR OF CHAGRIN FALLS & DINNER

DAY 3: WEDNESDAY

9:00 A.M.

Meet in the Hotel Lobby for Transportation to CTL

9:30 A.M. – 9:45 A.M.   LECTURE

NK/ADCC Target Cell Visualization Assay (NK-TVA™)
presented by Dr. Srividya Sundararaman

9:45 A.M. – 10:15 A.M.   LIVE DEMO

Performance of a cytolytic NK-TVA™ Assay
presented by Dr. Srividya Sundararaman
- Practice: Thawing cells, plating at different E:T ratios

10:15 A.M. – 11:15 A.M.   LECTURE

Measuring the antigen-specific T cell classes: CD8 effector vs. CD8 memory for CD4 Th1, Th2, Th17, and polyfunctional T cells
presented by Prof. Dr. Paul V. Lehmann

11:15 A.M. – 11:30 A.M   COFFEE BREAK

11:30 A.M. – 12:00 NOON   LECTURE

Using the antigen dose response to measure the affinity of the antigen-specific T cell response
presented by Prof. Dr. Paul V. Lehmann

12:00 NOON – 12:30 P.M. LIVE DEMO / TUTORIAL / HANDS-ON

Analysis of the three-color T cell assay performed the day before
presented by Jodi Hanson
- Polyfunctional cells
- Linearity of coexpressor frequencies

12:30 P.M. – 1:30 P.M.   LUNCH BREAK

1:30 P.M. – 1:45 P.M.   LECTURE

Characterizing Th1, Th2, Th17 and Polyfunctional HCMV-specific T cells
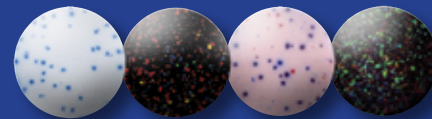*presented by Prof. Dr. Paul V. Lehmann*

1:45 P.M. – 2:30 P.M.   LECTURE

The choice of antigen for T cell immune monitoring
presented by Prof. Dr. Paul V. Lehmann

2:30 P.M. – 2:45 P.M.   COFFEE BREAK

2:45 P.M. – 3:00 P.M.   LECTURE

CPI protein pool as a positive control for the detection of functional CD4 T cells in human PBMC
presented by Prof. Dr. Paul V. Lehmann

3:00 P.M. – 4:00 P.M.   LIVE DEMO / TUTORIAL / HANDS-ON

Evaluation of the cytolytic NK-TVA™ Assay initiated in the morning
presented by Dr. Srividya Sundararaman & Villian Naeem

5:30 P.M.   UNIVERSITY CIRCLE CONCERT, DINNER

DAY 4: THURSDAY

9:00 A.M.

Meet in the Hotel Lobby for Transportation to CTL

9:30 A.M. – 10:15 A.M.   LIVE DEMO

Performance of a four-color B cell ELISPOT assay
presented by Richard Caspell

Covering:
- Theory
- Practice: Thawing cells, plating

10:15 A.M. – 10:45 A.M.   LECTURE

The unique features of B cell ELISPOT for immune monitoring
presented by Prof. Dr. Paul V. Lehmann
- Detection of B cell memory vs. active B cell response (plasmablasts)

10:45 A.M. – 11:00 A.M   COFFEE BREAK

11:00 A.M. – 11:30 A.M.   LECTURE

Why B cell ELISPOT #1 — Sensitivity: HCMV exposure revealed by B cell that serodiagnostic failed to detect
presented by Prof. Dr. Paul V. Lehmann

11:30 A.M. – 12:00 NOON   LECTURE

Why B cell ELISPOT #2 — Diagnostic: Detecting B cell autoimmunity in Multiple Sclerosis permitting diagnosis of the disease and monitoring of its activity
presented by Prof. Dr. Paul V. Lehmann

12:00 NOON – 1:00 P.M.   LUNCH BREAK

1:00 P.M. – 1:45 P.M.   LECTURE

Novelty: ELISPOT for rapid assessment of the humoral immune response's affinity spectrum. Morphology and kinetics of B cell ELISPOT formation permits measuring the affinity for antigen of the individual antibody-secreting B cells
presented by Dr. Alexey Karulin

1:45 P.M. – 2:00 P.M.   LIVE DEMO

ICS output and analysis of B cell affinities
presented by Richard Caspell

2:00 P.M. – 2:15 P.M.   COFFEE BREAK

2:15 P.M. – 2:45 P.M.   LECTURE

Harmonization of ELISPOT: Introduction
presented by Prof. Dr. Paul V. Lehmann

2:45 P.M. – 3:00 P.M.   LECTURE

Successful harmonization: Even first-time users in multi-center study have obtained highly-reproducible ELISPOT results when adhering to the CTL Protocol
presented by Jodi Hanson

3:00 P.M. – 3:30 P.M.   LECTURE

Multi-center study showing different investigators obtaining the same spot counts when gating with the ImmunoSpot® SmartCount™ module, but variable counts when setting gates manually according to their best judgment
presented by Richard Caspell

3:30 P.M. – 4:15 P.M.   LECTURE

Morphometric analysis of macroscopic bioassays
presented by Prof. Dr. Paul V. Lehmann

4:00 P.M. – 4:30 P.M.   LIVE DEMO / HANDS-ON

Analysis of viral plaque and colony forming assays
presented by Tameem Ansari

5:30 P.M.   DINNER CRUISE ON THE NAUTICA QUEEN

DAY 5: FRIDAY

9:00 A.M.

Meet in the Hotel Lobby for Transportation to CTL

9:30 A.M. – 10:15 A.M.   LIVE DEMO

Analysis of four-color B cell ELISPOT assay performed the day before
presented by Richard Caspell

10:15 A.M. – 10:30 A.M.   HANDS-ON

Analysis of four-color B cell ELISPOT assay performed the day before
presented by Richard Caspell, Jodi Hanson, Tameem Ansari & Villian Naeem

10:30 A.M. – 11:00 A.M.   LECTURE

Novel applications of ImmunoSpot® fluorescent readers in fluorescence microscopy and single-cell analysis
presented by Dr. Alexey Karulin

11:00 A.M. – 11:30 A.M.   LECTURE

Single-cell imaging with fluorescent ImmunoSpot® Analyzers
presented by Dr. Srividya Sundararaman

11:30 A.M. – 12:00 NOON   Q&A

led by Prof. Dr. Paul V. Lehmann

12:00 NOON – 2:00 P.M.   LUNCHEON / AWARDS CEREMONY