

# PEARLS OF LABORATORY MEDICINE

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**TITLE: Enumeration of CD34+ Stem Cells by Flow Cytometry**

**PRESENTER: Dustin Strasburg, MLS(ASCP)<sup>CM</sup>**

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**Slide 1:**

Hello, my name is Dustin Strasburg. I am a Development Technologist for the Human Cellular Therapy Laboratory at Mayo Clinic. Welcome to this Pearl of Laboratory Medicine on the **“Enumeration of CD34+ Hematopoietic Progenitor Cells by Flow Cytometry.”**

**Slide 2:**

Hematopoietic Progenitor Cells or HPCs are used to reconstitute hematopoiesis following marrow-ablative therapies. Traditional colony-forming assays take 10-14 days and are thus irrelevant when immediate assessment of HPC products, such as apheresis, is necessary. Flow cytometry is used to enumerate CD34+ HPCs to quickly determine the potency of a collected HPC product. However, these cells are rare events which may be masked by nonspecific or interfering populations. Furthermore, various methodologies led to a wide range of recommended CD34 values needed for long-term engraftment.

In 1995, the International Society of Hematotherapy and Graft Engineering (ISHAGE) developed a simple and sensitive method that utilized the CD34 antigen and several other characteristics. By using only 4 parameters and sequential gating to minimize interference, this assay can be performed by most routine clinical flow cytometry laboratories.

**Slide 3:**

The ISHAGE criteria for CD34+ HPCs include: expression the CD34 antigen, expression the CD45 antigen but characteristically at lower levels, low side scatter, and low to intermediate forward scatter. The scatter properties are similar to but slightly higher than small lymphocytes, which can be used as an aid in accurate gating.

**Slide 4:**

Furthermore, a viability marker such as 7-amino actinomycin (or 7-AAD) allows for the exclusion of dead cells. The use of fluorescent counting beads with a known concentration is used to determine an absolute concentration of CD34+ HPCs.

## Slide 5:

The first gate is set around the white blood cells which are CD45+. This gate excludes CD45-negative contaminants such as platelets, platelet aggregates, non-lysed red blood cells, and other debris. It should not to exclude CD45<sub>dim</sub> events as this would exclude potential HPCs prior to further analysis. A gate is also set around the lymphocyte cluster to be used later.

## Slide 6:

Using the WBC events, a gate is set around all potential CD34+ events.

## Slide 7:

Looking at only the CD34+ events, the target cell population can be identified more easily. A gate is placed around the cell cluster. This cluster exhibits lower CD45 expression than typical WBCs with low side scatter, similar to lymphocytes.

## Slide 8:

Utilizing the scatter properties of lymphocytes gated earlier, a linked or mirrored gate is set around the HPC population. In this example, there are a large number of HPC events; however, many times this population is more diffuse and not as easily identified. Events falling within this gate exhibit all 4 parameters to be classified as CD34+ Hematopoietic Progenitor Cells.

## Slide 9:

Fluorescent beads can be assessed with any parameter. This example used the FL3 channel plotted across time to select the singlet beads. Exclusion of doublets is critical in accurate analysis of the bead concentration.

Events that meet the 4 ISHAGE parameters are then assessed for viability. Viable cells are able to exclude the dye and thus appear negative for the marker 7-AAD.

## Slide 10:

To calculate the CD34+ HPC concentration, we take the ratio of viable CD34+ HPC events to the number of singlet bead events and multiply that by the CAL factor (or known bead concentration).

In this example, the sample or product would have 121.7 viable CD34 cells/ $\mu$ L.

## Slide 11:

In summary, the ISHAGE protocol is a single-platform flow assay that determines the absolute concentration of viable CD34+ Hematopoietic Progenitor Cells in a sample or product. This value can be used with the volume of product to determine the total CD34+ HPC content and further calculated to determine the dose per patient body weight of an HPC product.

## **Slide 12: References**

## **Slide 13: Disclosures**

## **Slide 14: Thank You from [www.TraineeCouncil.org](http://www.TraineeCouncil.org)**

Thank you for joining me on this Pearl of Laboratory Medicine on “**Enumeration of CD34+ Hematopoietic Progenitor Cells by Flow Cytometry.**”



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## PEARLS OF LABORATORY MEDICINE

### **Enumeration of CD34+ Hematopoietic Progenitor Cells by Flow Cytometry**

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Department of Laboratory Medicine and Pathology

DOI:



# CD34+ HPC by Flow Cytometry

- Reconstitute long-term multilineage hematopoiesis following marrow-ablative therapies
- Flow cytometry used to quickly determine potency of HPC products
  - Colony-forming assays take 10-14 days
  - Rare events masked by interfering populations
- 1995: ISHAGE developed simple method
  - Utilize CD34 antigen characteristic of HPC
  - 4-parameters (light scatter and 2-color immunofluorescence)
  - Sequential gating minimizes interference of nonspecific staining

# CD34+ HPC by Flow Cytometry

- 1) Express CD34 antigen
- 2) Express CD45 antigen like blasts
  - Positive but at low levels (i.e. CD45<sub>dim</sub>)
- 3) Low side scatter
- 4) Low to intermediate forward scatter
  - Slightly higher than small lymphocytes



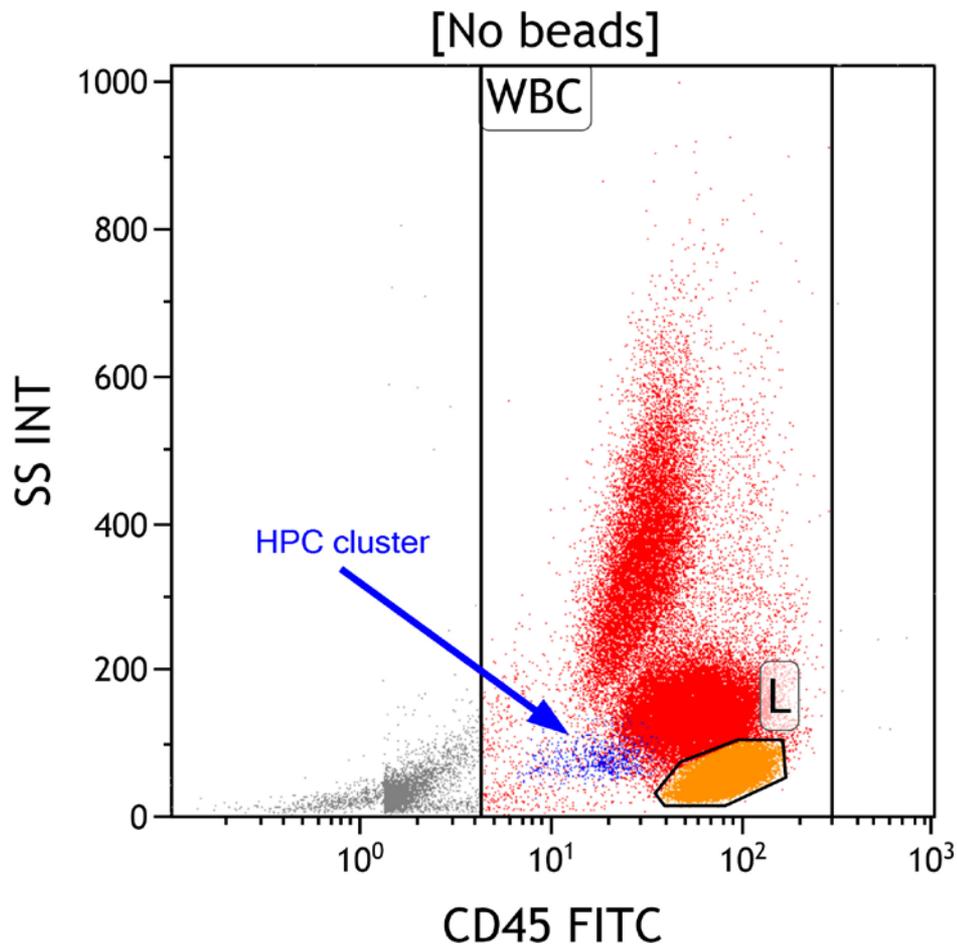
# CD34+ HPC by Flow Cytometry

- Viability marker (e.g. 7-AAD) allows the exclusion of dead cells
- Use of fluorescent counting beads to calculate absolute concentration (single-platform)



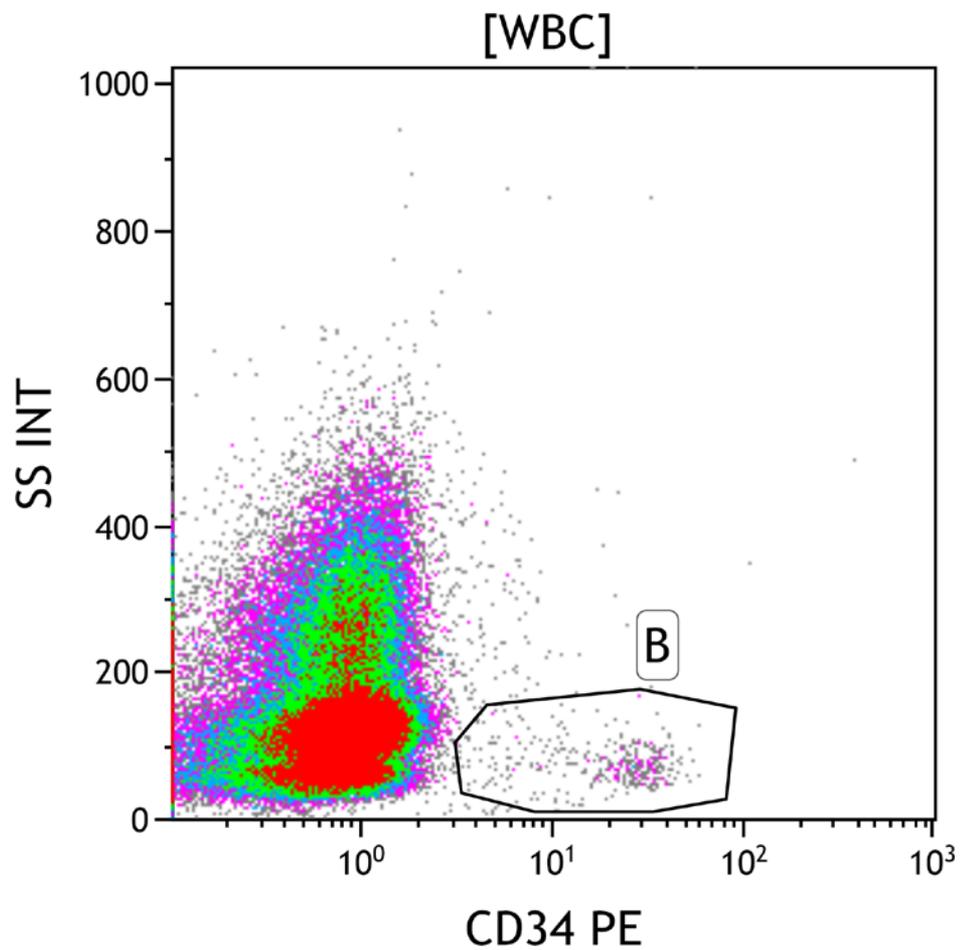
## CD45 vs Side Scatter (Cellular events)

- Exclude contaminants such as platelets/aggregates, non-lysed RBCs and other debris
- Exclude CD45 negative events while taking care not to exclude HPCs which are CD45<sub>dim</sub>
- Identify lymphocyte cluster (L) to be used later



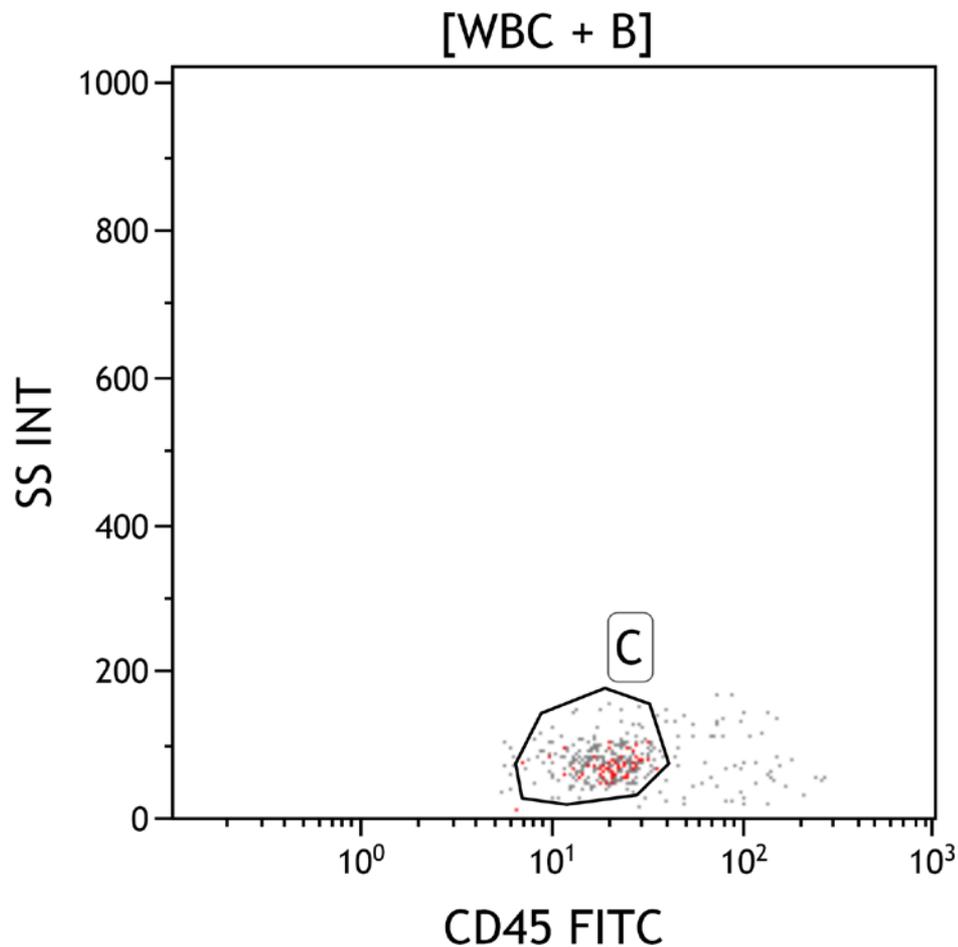
## CD34 vs Side Scatter (WBC events)

- Used to include all potential CD34+ cells



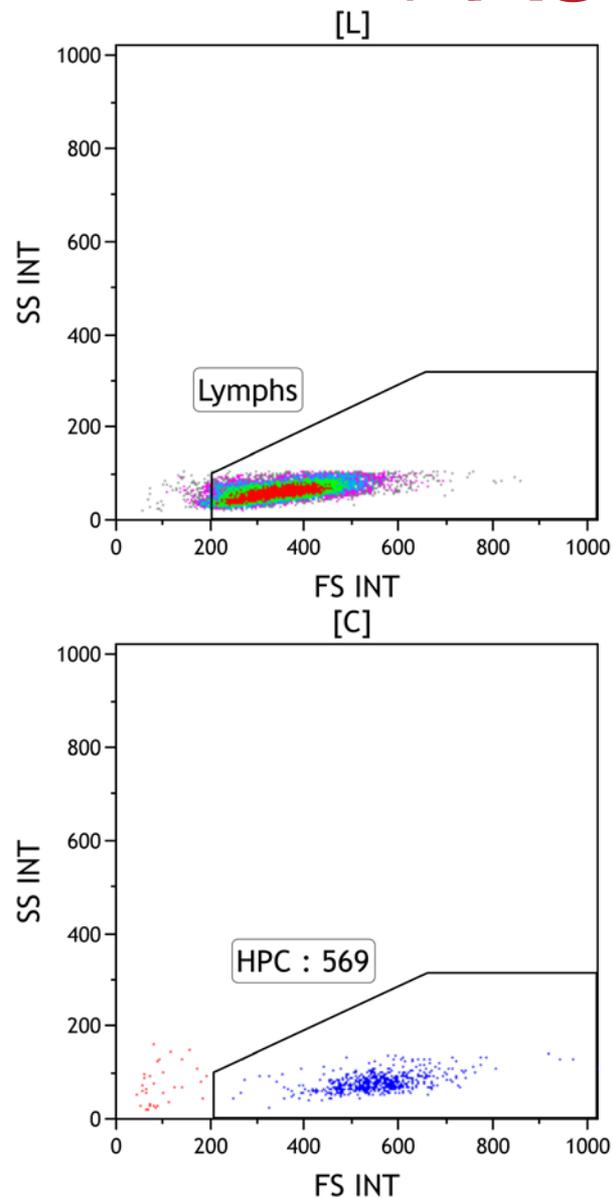
## CD45 vs Side Scatter (CD34+ events)

- Identify the HPC cell cluster
- Lower CD45 expression than typical WBC
- Low Side Scatter, similar to Lymphocytes



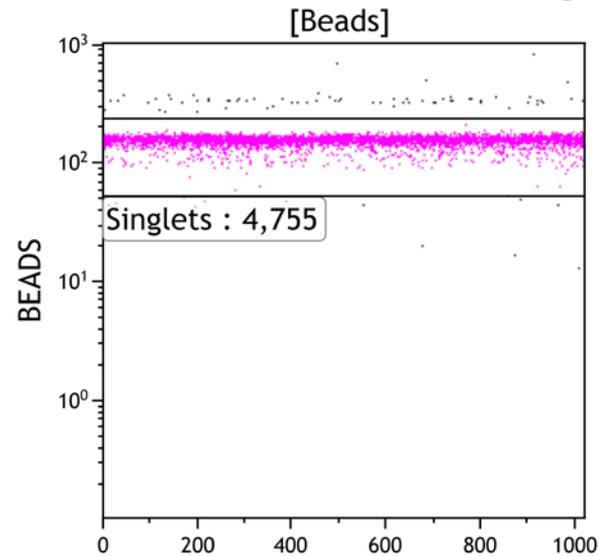
## Forward vs Side Scatter (Top: Lymphocytes) (Bottom: CD34 cell cluster)

- Use Lymphocyte gate as a guide
- Mirrored HPC gate excludes platelets/aggregates that may stain positively CD45/CD34
- Events in this gate meet all 4 parameters to be classified as CD34+ Hematopoietic Progenitor Cells



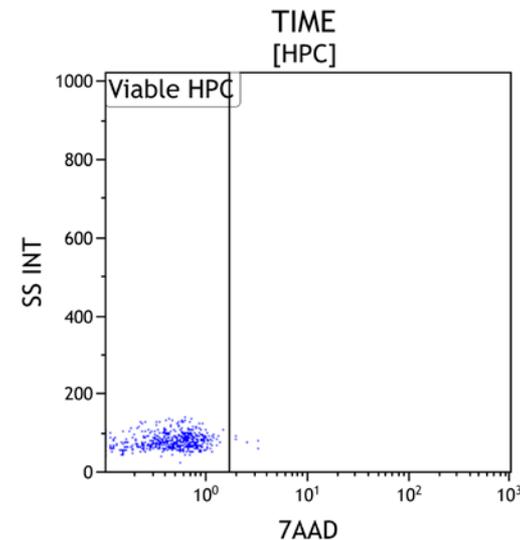
## Time vs FL3 (Bead events)

- Select singlet bead events
- Ratio of bead to cell events used to calculate concentration



## 7-AAD vs Side Scatter (HPC events)

- Viable cells exclude the dye and appear negative for the marker



Gate	Number	%Gated
All	569	100.00
Viable HPC	564	99.12

## Calculation of CD34+ HPC Concentration

$$\frac{\# \text{ of viable HPC events}}{\# \text{ of singlet bead events}} \times \text{CAL factor}$$

Example:

$$\frac{564 \text{ (viable CD34 cells)} \times 1026 \text{ (beads/uL)}}{4,755 \text{ (beads)}} \\ = \mathbf{121.70 \text{ viable CD34 cells/uL}}$$

NOTE: Multiply result by any dilution factor

# Summary

- ISHAGE protocol is a single-platform flow assay that determines the absolute concentration of viable CD34+ HPCs in a sample or product
  - Calculation of HPC content in a product
  - Calculation of HPC dose per patient body weight



# References

1. Sutherland D.R., Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. *J Hematother* 1996;5:213-26.
2. Sutherland D.R., Keeney M. Enumeration of CD34+ Cells by Flow Cytometry. *Cellular Therapy: Principles, Methods and Regulations. The American Association of Blood Bankers (AABB)* 2016:558-69.
3. Keeney M, Chin-Yee I, Weir K, et al. Single Platform Flow Cytometric Absolute CD34+ Cell Counts Based on the ISHAGE Guidelines. *Cytometry* 1998;34(2):61-70.

**Please provide full citations for your references in style with Journal format as explained here:**

<http://clinchem.aaccjnls.org/content/information-authors#References>



# Disclosures/Potential Conflicts of Interest

*Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:*

- **Employment or Leadership:**
- **Consultant or Advisory Role:**
- **Stock Ownership:**
- **Honoraria:**
- **Research Funding:**
- **Expert Testimony:**
- **Patents:**

**\*\*\*This slide will be completed by CCJ Staff based on disclosure form completed electronically in the submission site.\*\*\***



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**QUESTION BANK TEMPLATE**

Field	Instructions	
Stem	Write one question <i>Refer to Guide for Presenters for guidance (Page 5)</i>	What is the characteristic antigen found on Hematopoietic Progenitor Cells?
Responses	Provide 5 responses <i>Refer to Guide for Presenters for guidance (Page 5)</i>	CD45 CD34 HLA-DR CD3 CD4
Answer	Indicate one correct response	CD34
Discussion	Provide a discussion of the correct response with main points explaining why it is the best choice	CD34 is a characteristic antigen found on Hematopoietic Progenitor Cells. It is rarely found on other cell populations and, therefore, used to identify HPCs.
Source(s)	Provide the source(s) of information for further study <i>Refer to Guide for Presenters for full citation formatting (Page 3)</i>	Sutherland D.R., Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. J Hematother 1996;5:213-26.
Difficulty	Select one level of difficulty: <i>Easy, intermediate, advanced</i>	Easy
Category	Select one category ( <i>Refer to list in Guide for Presenters - Page 6</i> )	Transfusion Medicine
Sub-category	Select one sub-category ( <i>Refer to list in Guide for Presenters - Page 6</i> )	Transfusion Medicine-Other
Keywords	Include at least 1-2 keywords <i>Keywords should describe a subtopic to the sub-category selected. Examples include, thyroid, electrolytes, diabetes, pregnancy, etc.</i>	HPC, Stem cells

## Question Bank Template

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Field	Instructions	
Stem	Write one question <i>Refer to Guide for Presenters for guidance (Page 5)</i>	What gating strategy is used to sequentially eliminate non-specific events when enumerating CD34+ stem cells by flow cytometry?
Responses	Provide 5 responses <i>Refer to Guide for Presenters for guidance (Page 5)</i>	Stem Cell 3D scatter analysis ISHAGE Linked Isotype Controls Fluorescence Minus One
Answer	Indicate one correct response	ISHAGE
Discussion	Provide a discussion of the correct response with main points explaining why it is the best choice	The ISHAGE guidelines utilizes 4 parameters and sequential gating to select the population of interest while simultaneously eliminate interfering populations.
Source(s)	Provide the source(s) of information for further study <i>Refer to Guide for Presenters for full citation formatting (Page 3)</i>	Sutherland D.R., Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. J Hematother 1996;5:213-26.
Difficulty	Select one level of difficulty: <i>Easy, intermediate, advanced</i>	Intermediate
Category	Select one category ( <i>Refer to list in Guide for Presenters - Page 6</i> )	Transfusion Medicine
Sub-category	Select one sub-category ( <i>Refer to list in Guide for Presenters - Page 6</i> )	Transfusion Medicine-Other
Keywords	Include at least 1-2 keywords <i>Keywords should describe a subtopic to the sub-category selected. Examples include, thyroid, electrolytes, diabetes, pregnancy, etc.</i>	Gating, nonspecific