From: Aleksandr Rabodzey
To: Brian Grossman; Vivek Khanna
Sent: 1/24/2014 7:03:47 AM
Subject: Theranos
Attachments: method validation_tcm45-9295.pdf; Parvovirus IgG assay variation.pdf; PattiJonesValidationCaseStudieswoanswers.pdf; UCM172009.pdf

Here are the reference documents for our discussion today

1. Deck on validation and I pasted the slide with suggested CV of 5-10%
2. Parvovirus IgG assay with 4% CV vs Theranos at 15-20% (see p143 of their presentation)
3. I also attached two more data presentation with illustrative graphs of R2 for commonly used tests. Most of them are >95% vs Theranos usually under 95% with the exception of simple general chemistry

Basically, my concern is that their methodology is still raw and/or they may be pushing limits here. I do not think it's a deal breaker as long as they can get CLIA and FDA approval on their tests AND can maintain certification in future. Note, that they will have to re-run these tests quarterly, and LH/DGX will eat them alive if there is any evidence of 20% CV here.

Good example of what I am referring to is p137 the LH assay. Their R2 is pretty poor at .91 and there are very few data points at the higher end of the range. This compares to 0.992 R2 for Leinco LH test on 110 samples vs their ~20
http://www.leinco.com/ELISA-Kit/Luteinizing-Hormone/pdf/T110.pdf

Basic Definitions: Coefficient of Variation

- Coefficient of Variation (C.V.) is a way to express imprecision.
- C.V. = standard deviation ÷ mean
- Although, strictly, the C.V. can vary with the magnitude of the value, it is often used as if it doesn't. Hence, in this way it expresses a proportional variability. Across a limited range this assumption is probably warranted.
- In general, coefficients of variation should be below 5%, and should rarely exceed 10%, across the medically-relevant range of analyte concentrations.

Validation of New Methods

Laboratory Medicine Residency Didactic Conference
June 10, 2003

Sources

- Chapter 13, *Tietz Textbook of Clinical Chemistry*, 3rd ed.
- "Toxicology Assay Validation Procedure", Clinical Chemistry Laboratory, YNHH, written by Petrie Rainey, November, 1995.
- The Westgard Web Lessons: www.westgard.com/lesson.htm
- "Method Performance Specifications" section of the CAP "Laboratory General Checklist" (www.cap.org)
- CLIA-88, Subpart K, 493.1213 Standard; "Establishment and verification of method performance specifications"; refer to www.vh.org/adult/provider/pathology/CLIA/CLIAHP.html

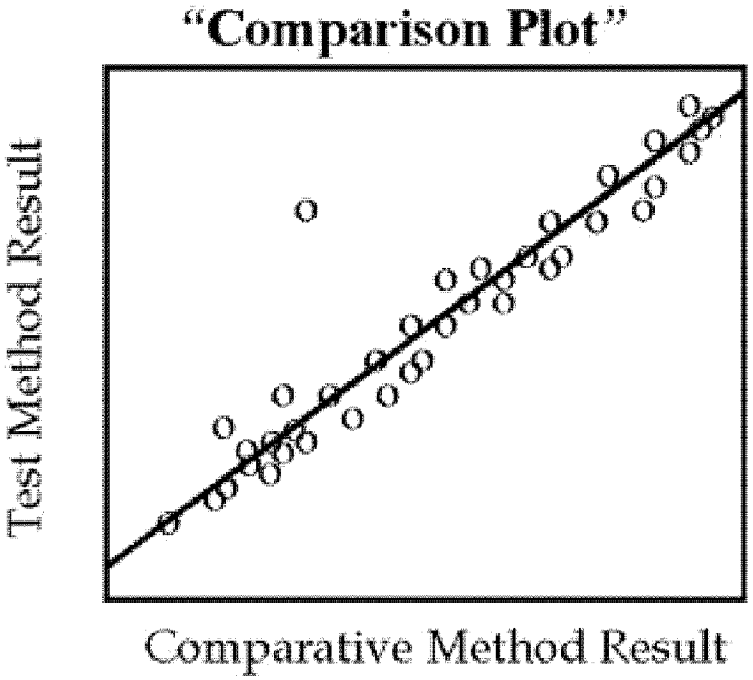
Basic Definitions: Accuracy

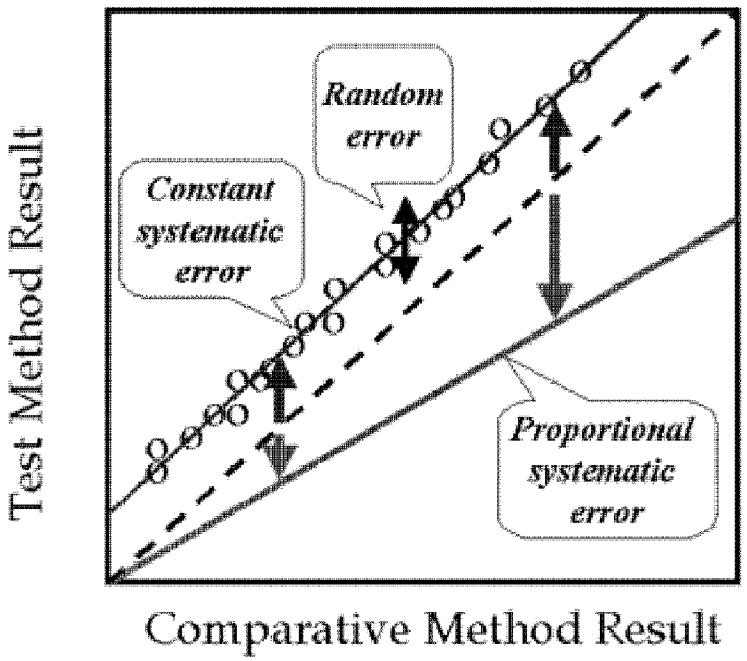
- Defined as "the closeness of the agreement between the measured value of an analyte and its 'true' value."
- Of course, the catch is defining a "true" value.
- A number of organizations exist to define and provided standardized reference materials:
  - National Reference System for the Clinical Laboratory (NRSCL)
  - The Standards Committee of the AACC
  - National Institute of Standards and Technology (NIST)
- Accuracy is often expressed using the concept of *error*.

Basic Definitions: Systematic Error

- A measure of the agreement between the average measured value and the "true" value.
- Best described by a correlation plot between measured and reference values.
- Can be divided into two categories
  - *Constant Systematic Error*: Manifests as a constant difference between the measured and true values and corresponds to the "vertical offset" of a correlation plot. Often caused by an interfering substance. Can be practically eliminated by a proper "blank" as long as the interference is constant.
  - *Proportional Systematic Error*: Varies with the magnitude of the value, often linearly, and corresponds to a non-unity slope of the correlation plot. Generally reflects a failing of the methodology. Sometimes, may be simply fixed by recalibration and, other times, may be more insidious.
- "Bias" is defined simply as the error expressed as a percent.

Correlation/Comparison Plot

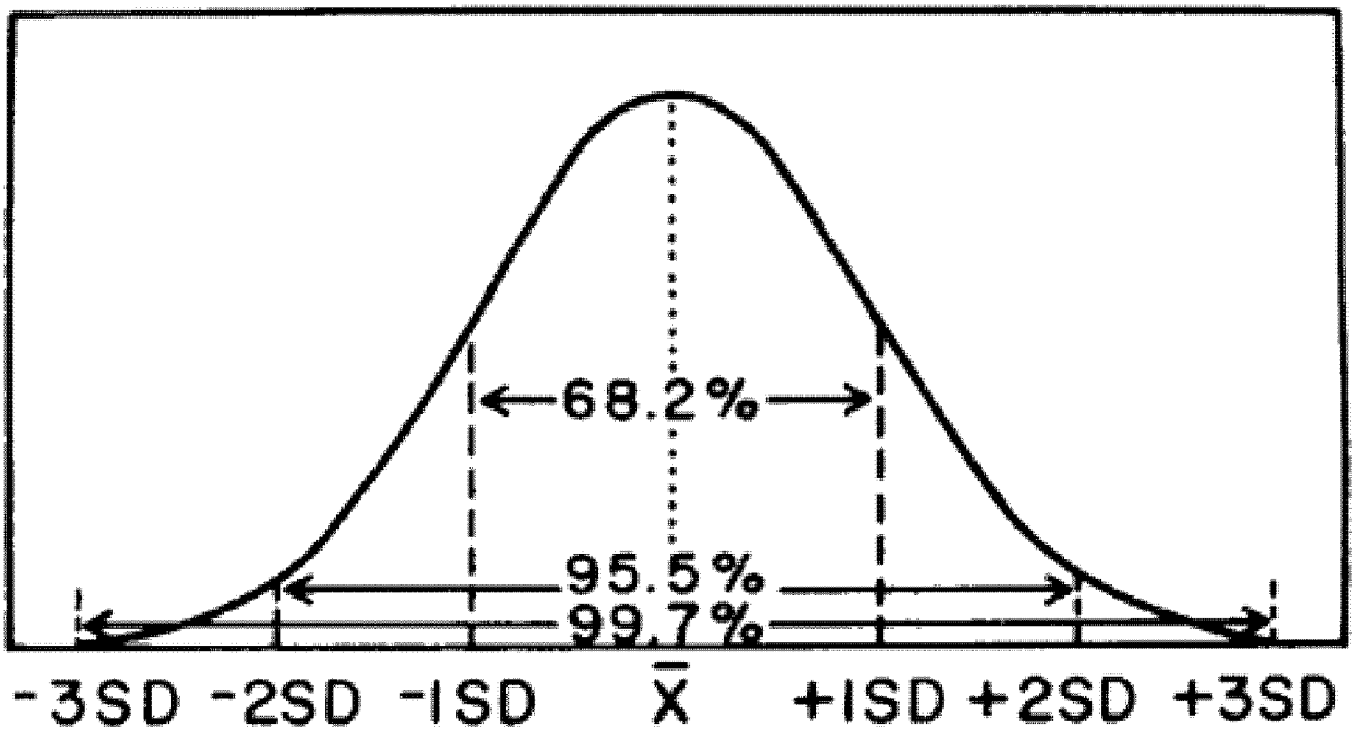




Basic Definitions: Random Error

- Fluctuations of the measured values about their mean due to random factors.
- An indicator of *analytical precision.*
- In a correlation plot, reflected by the deviations of the measured values from a straight line.
- When conforming to a Gaussian or Normal distributions, these fluctuations are mathematically encapsulated by the *standard deviation,* which can be used to predict statistical probabilities.
- Although not strictly a component of analytical accuracy, random error can be considered to contribute to the "correctness" of a reported result and is included in the *total error.*

Gaussian Distribution



Basic Definitions: Total Error

- Sum of the systematic error and the random error (2 – 4 times the standard deviation of the imprecision).
- Meant to be a "worst case" incorrect answer.
- As both the systematic and the random error can be value-dependent, it is necessary to define the total error at each of the important parameter values (i.e. at the clinical decision points) and compare to the pre-defined medical requirements for the assay.

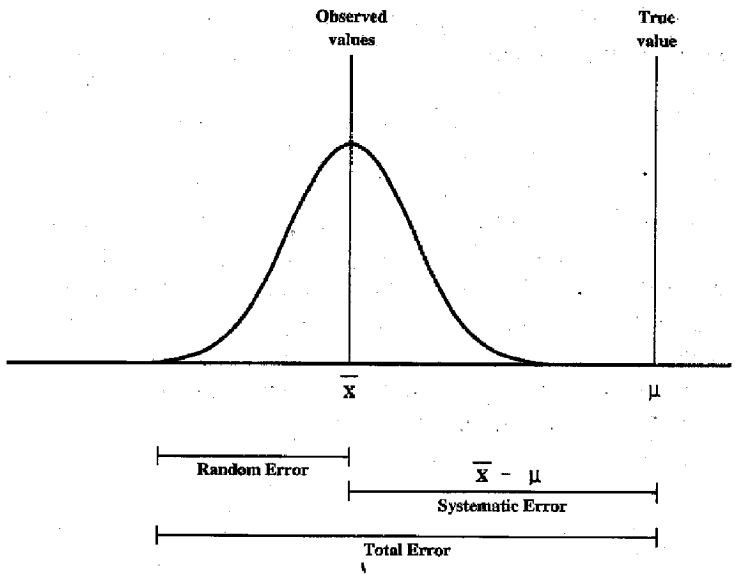


FIGURE **13-3.** The total error concept of accuracy. (Modified from Westgard, J.O., de Vos, D.J., Hunt, M.R., et al.: Method Evaluation. Houston, American Society for Medical Technology, 1978.)

Basic Definitions: Precision

- Same as random error.
- Always basically determined by repeated analysis of samples with expected "correct" answers.
- Three categories:
  - Within-run precision
  - Between-run or within-day precision
  - Day-to-day precision (best estimate of total precision and for calculation of the total error)

Basic Definitions: Coefficient of Variation

- Coefficient of Variation (C.V.) is a way to express imprecision.
- C.V. = standard deviation ÷ mean
- Although, strictly, the C.V. can vary with the magnitude of the value, it is often used as if it doesn't. Hence, in this way it expresses a proportional variability. Across a limited range this assumption is probably warranted.
- In general, coefficients of variation should be below 5%, and should rarely exceed 10%, across the medically-relevant range of analyte concentrations.

Steps Required to Validate an Assay (in no particular order)

- Establish Accuracy
- Establish Precision
- Establish the Reportable Range (linear range)
- Determine the Analytical Sensitivity and the Lower Limit of Detection
- Investigate the Analytical Specificity (search for interferences)
- Establish the Reference Range (define medically relevant decision points).

Evaluation of Accuracy

1. Comparison with Reference Standards
   - Based on repeated analysis of multiple established standard solutions, which can be purchased or prepared locally, across a range of analyte concentrations. Expected values based on a reference or "gold standard" method.
   - Standard samples may be aqueous (common) or serum (preferred) based.
   - Make a correlation plot and fit to a straight line (if appropriate). Look for both constant and proportional systematic error.
2. Recovery Experiment
   - Known amounts of the analyte are added to samples containing an unkown amount of the analyte. Measurements are taken with and without the added analyte. The difference between the measurements is compared to the expected value as an indicator of "recovery".
   - This is typically performed across a range of concentrations and analyzed similarly to a correlation plot, above.
3. Correlation with a Current or Accepted Method
   - Prepared or clinical (preferred) samples are simultaneously analyzed by the two methods (i.e. the new and the old) and compared.
   - This step is absolutely required if replacing an existing method.
   - Differences may represent inaccuracies with either the old or new method and may need to be considered when establishing the new reference range.

Evaluation of Precision

- Need to get a sense of both within-run and day-to-day imprecision, at different analyte concentrations.
- For example, using a series of serum standards selected around medically important decision levels, independently assay five aliquots of each on at least five different days.
- Express variations in terms of coefficient of variation (within-run and day-to-day), bias and total error.
- The day-to-day C.V. should be ideally < 5 % and certainly no worse than 10 %, except at very low levels.
- The bias should not exceed the C.V. (implies a limitation of the assay that is probably correctable).
- The total error should be less than the acceptable performance standard for (CAP) proficiency testing.

Evaluation of the Reportable Range

- Although not strictly defined, generally implies establishing the *linearity range* for an assay.
- Non-linear assays are allowed, in which case this would translate into the region that fits the non-linear function used to describe the data.
- Evaluate by diluting standards and comparing the measured result to the expected result.
- The reportable range is defined by the highest and lowest points that fall within 1 C.V. of a straight line (or equivalent non-linear curve).
- CLIA-88 requires that the reportable range does not exceed the range of the available calibrators.

Determination of Analytical Sensitivity and the Lower Limit of Detection

- The analytical sensitivity is defined as the incremental increase in measured signal per incremental increase in analyte concentration.
- Analytical sensitivity is simply described by the slope of the calibration curve.
- An assay with a high analytical sensitivity *should* have a low limit of detection, but not necessarily.
- Hence, one way to estimate the lower limit of detection would be to estimate the minimal detectable increment in signal and calculate the corresponding concentration of the analyte.
- However, this would be inadequate as other factors are probably more important (lower limit of linearity, non-specific signals from blank samples, increased random error at low analyte concentrations, etc.)

Lower Limit of Detection, cont.

- The lower limit of detection should be investigated with three different approaches; the results then compared and the most sensible limit chosen.
- The first two choices would be (1) the lower limit of linearity and (2) minimal detectable analytical signal or the limit of quantification (where the C.V. exceeds 20%).
- The third method is to prepare a series of clinically-relevant blank samples (if possible) and measure the effective analyte concentration. The lower limit of detection would then be defined as the mean plus three standard deviations (such that there would be less than a 0.3 % chance of confusing analytical noise with a "true" measurment).

Evaluation of Analytical Specificity

- The effect of common analytical interferences, including plasma hemoglobin, bilirubin, lipemia, etc., must be established.
- Generally, serum is sought with high levels of the above potential interferents and then either
  - (i) the analyte can be added to the samples and a recovery experiment performed or
  - (ii) equal amounts of the samples can be mixed with samples containing pre-measured amounts of the analyte and a mixing study performed.
- Additionally, for every assay, the director should consider any other potential interferent. For example, for TDM, other structurally related drugs should be tested for interference in the assay.

Establishment of the Reference Range

References may be established in any number of several ways and is a major topic by itself (Chapter 14 in Tietz):

1. Direct determination of the reference range in the laboratory by monitoring analyte values in an appropriate population.
2. Use of a reference range established elsewhere, either based upon the medical literature, test manufacturer or within another clinical laboratory. Must somehow validate the appropriateness of this reference range locally (sample comparison, clinical study, etc.).
3. Establishment of the continued validity of a previous reference range used for an alternative method within the same local laboratory (i.e. "transfer" of the reference range from the old method to the new one).

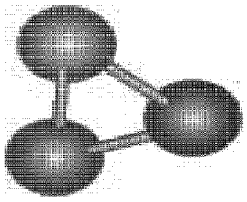
21st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)
27th International Congress of Chemotherapy (ICC)
07.05.2011 - 10.05.2011

Diagnostic/laboratory methods (other than molecular)

Evaluation of Liaison® Biotrin Parvovirus B19 kits for IgG and IgM antibody detection

A. Cardentey-Reyes*, C. Liesnard, M.L. Delforge (Brussels, BE)

Objectives: The purpose of this study was to compare the LIAISON® Biotrin Parvovirus B19 chemiluminescent immunoassay (CLIA) kit's performance to the Biotrin Parvovirus B19 Enzyme Immunoassay (EIA).
Methods: 273 serum samples were collected from patients with different antibody status according to Biotrin EIA: 112 IgG positive-IgM negative samples, 51 IgM positive samples and 110 IgG negative samples. Potential cross-reactions were evaluated using 45 serum samples from patients with other infectious diseases (n=30) or immunological pathologies (n=15). LIAISON® results were compared with those obtained with the Biotrin Parvovirus B19 EIA. Discordant results between LIAISON® and EIA were confirmed by Biotrin Parvovirus B19 Immunofluorescent Assay (IFA).
Results: Of the IgG positive-IgM negative group we found one sample was IgM positive by LIAISON®, negative by IFA. Of the IgM positive group, two sera were negative by LIAISON®. One was confirmed negative and one was weakly positive by IFA. Of the IgG negative group no discrepancies were found between the two tests. Of the potentially cross-reactive IgM samples, 2 were positive with LIAISON®, one of them was negative with EIA but both were positive with IFA. One showed doubtful results with the three methods. All those three false positive samples came from patients with acute CMV infection. Coefficients of variation for interassay variability for IgG and IgM antibodies were 4.5 % and 4 %, respectively.
Conclusions: The agreement between LIAISON® and Biotrin EIA was 100% for IgG. Concerning IgM we observed 3 discrepancies, of which 1 was confirmed by IFA. We can conclude that the fully automated LIAISON® Biotrin Parvovirus B19 CLIA kits showed a good agreement with Biotrin EIA for detection of IgG and IgM antibodies.



Professional Practice in Clinical Chemistry

Validation Case studies: the good, the bad and the molecular

Patti Jones PhD

Professor of Pathology

UT Southwestern Medical Center

Director of Chemistry

Children's Medical Center Dallas

Presented by AACC and NACB

Learning Objectives

- Through example validations:
  - Compare the assay validations required for FDA-approved tests versus Laboratory Developed Tests (LDTs)
  - Delineate the basic steps to validating an assay
  - Discuss the validation of a molecular test

Professional Practice in Clinical Chemistry

Method Validation

- Clinical Laboratory Improvement Amendments of 1988 (CLIA '88)
  - Subpart K – Quality System for Non-waived Testing
  - Sec. 493.1253 Standard: Establishment and verification of performance specifications

Tells you **what** must be validated,
but not **how** to do it.

Professional Practice in Clinical Chemistry

Method Validation

- CLIA Regulation 493.1253(2)
  1. Accuracy – (closeness to true/comparative method)
  2. Precision – (reproducibility)
  3. Reportable range – (linearity, AMR, MD/C)
  4. Reference interval –
  5. Analytical sensitivity – (lower limit)
  6. Analytical specificity – (interferences)
  7. Other specifications

Professional Practice in Clinical Chemistry

Case 1: the Good – FDA-approved methods/reagents

- CLIA Regulation 493.1253(2)
  1. Accuracy
  2. Precision
  3. Reportable range
  4. Reference interval

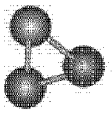
Determine the assay performs in your hands the way the manufacturer says it performs.

  5. Analytical sensitivity
  6. Analytical specificity
  7. Other specifications

Professional Practice in Clinical Chemistry

Case 1: FDA-approved test

- Three basic studies:
  - Precision studies
    - Reproducibility
  - Reportable Range Study
    - Linearity, analytical measurement range (AMR), maximum dilution/concentration (MD/C)
  - Assay comparison/correlation study
    - Accuracy, Reference interval



Professional Practice in Clinical Chemistry

Case 1: glucose

- Precision Studies:
  - Within-run precision
    - Patient or QC samples assayed 20 times on the same day within the same run
    - If precision poor, no need to do further eval
  - Between-run precision
    - Patient or QC samples once per day for 20 days
    - Establish qc range as well as total precision
  - Samples at least 2 - 3 levels – medical decision points

Professional Practice in Clinical Chemistry

Case 1: Glucose: precision

Table 1. Data on imprecision.

Sample	Glucose mg/dL (mmol/L)		CV, %
	Mean	**SD**	
Within run (n = 20 replicates)			
I	50.4 (2.8)	1.4 (0.08)	2.8
II	200.6 (11.14)	2.7 (0.15)	1.4
Between run (n = 20 runs)			
I	51.2 (2.84)	2.1 (0.12)	4.1
II	202.3 (11.24)	3.5 (0.19)	1.7

Case 1: Glucose: precision

- What's good precision?
  - Depends on the analyzer and the analyte
    - <5% CV considered good
    - Many automated analyzers (blood gases) < 1% CV
    - Tandem MS, HPLC, etc <10% CV is excellent

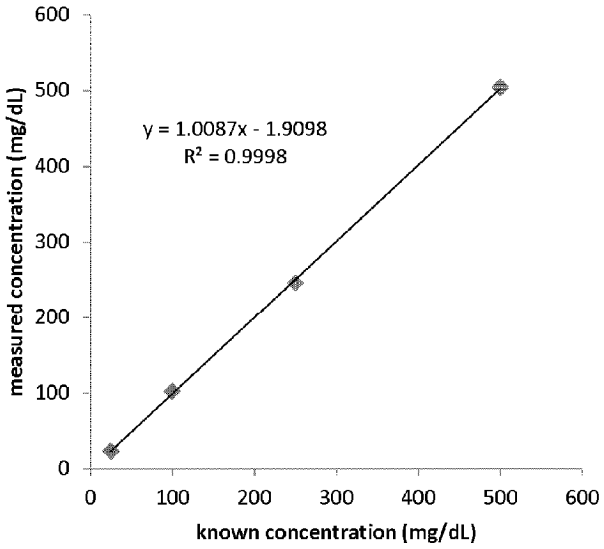
Professional Practice in Clinical Chemistry

Case 1: Glucose: reportable range

- Validation of Reportable Range
  - Minimum of 3 test specimens (4-5 better), measured in duplicate or triplicate
  - Appropriate matrix
  - Well established target concentrations
  - Concentrations near the low, midpoint, and high values of the AMR

Professional Practice in Clinical Chemistry

Case 1: Glucose : reportable range



known	Average measured
25	23
100	102
250	246
500	504

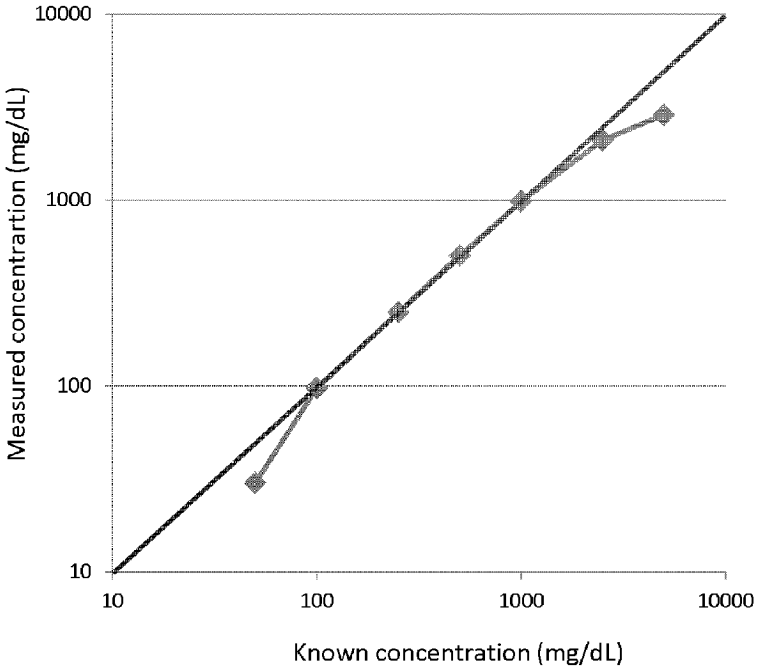
linearity = 25 – 500 mg/dL
CAP "AMR"

CLIA "reportable range" = CAP AMR +

Verify maximum dilution/concentration (MD/C) (CRR)?
• spike serum with high concentration (5000+)

Professional Practice in Clinical Chemistry

Case 1: glucose: reportable range - MD/C



dilution	theor	measure
1:100	50	30
1:50	100	97
1:20	250	248
1:10	500	502
1:5	1000	992
1:2	2500	2100
0	5000	2875

Professional Practice in Clinical Chemistry

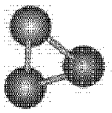
Case 1: Glucose: Accuracy - Correlation

- Comparison of Methods – correlation
  - Select a minimum of 20 (usually 40 – 60) patient's serum samples with analyte values as **evenly distributed throughout the linear reportable range of the assay** as possible
  - Assay all samples by the current method (comparative or reference method – x-axis data) and the method being evaluated (test method – y-axis data)

Professional Practice in Clinical Chemistry

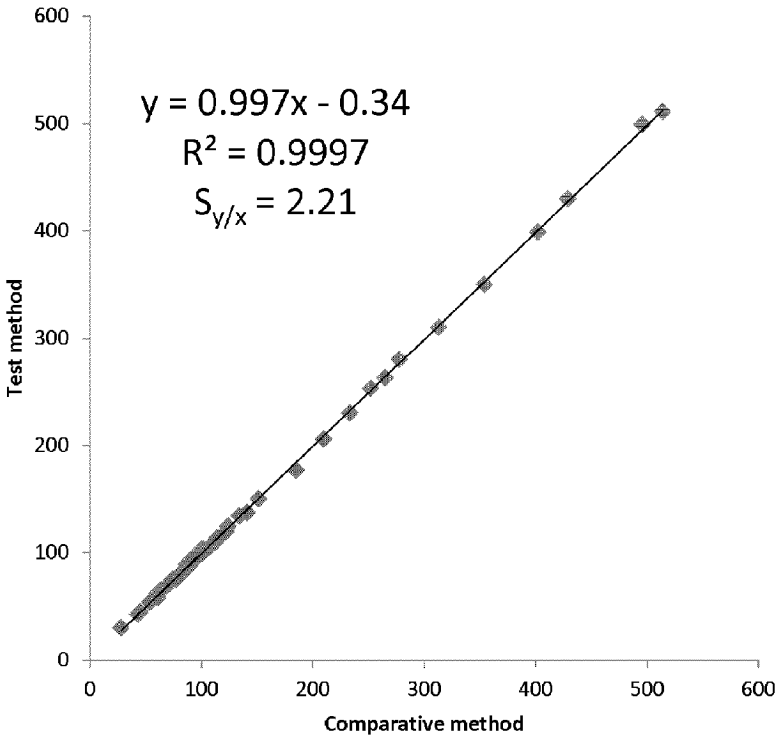
Case 1: Glucose - correlation

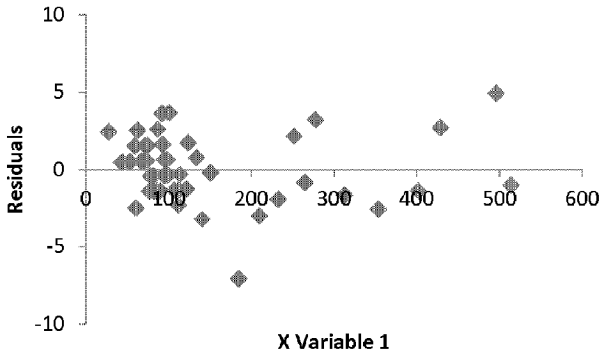
- Correlation experiment:
  - Plot (x,y) pairs of values and apply appropriate regression analysis to these data
  - Obtain linear regression equation of the line (least squares line)
    - Assumption: Any errors are in the test method (TM) and not the comparative method (CM)
  - Deming Regression – assigns errors to both methods depending on their variances
  - Slope, intercept, correlation coefficient, standard error of the estimate, bias plot



Professional Practice in Clinical Chemistry

Least-Squares Linear Regression Data

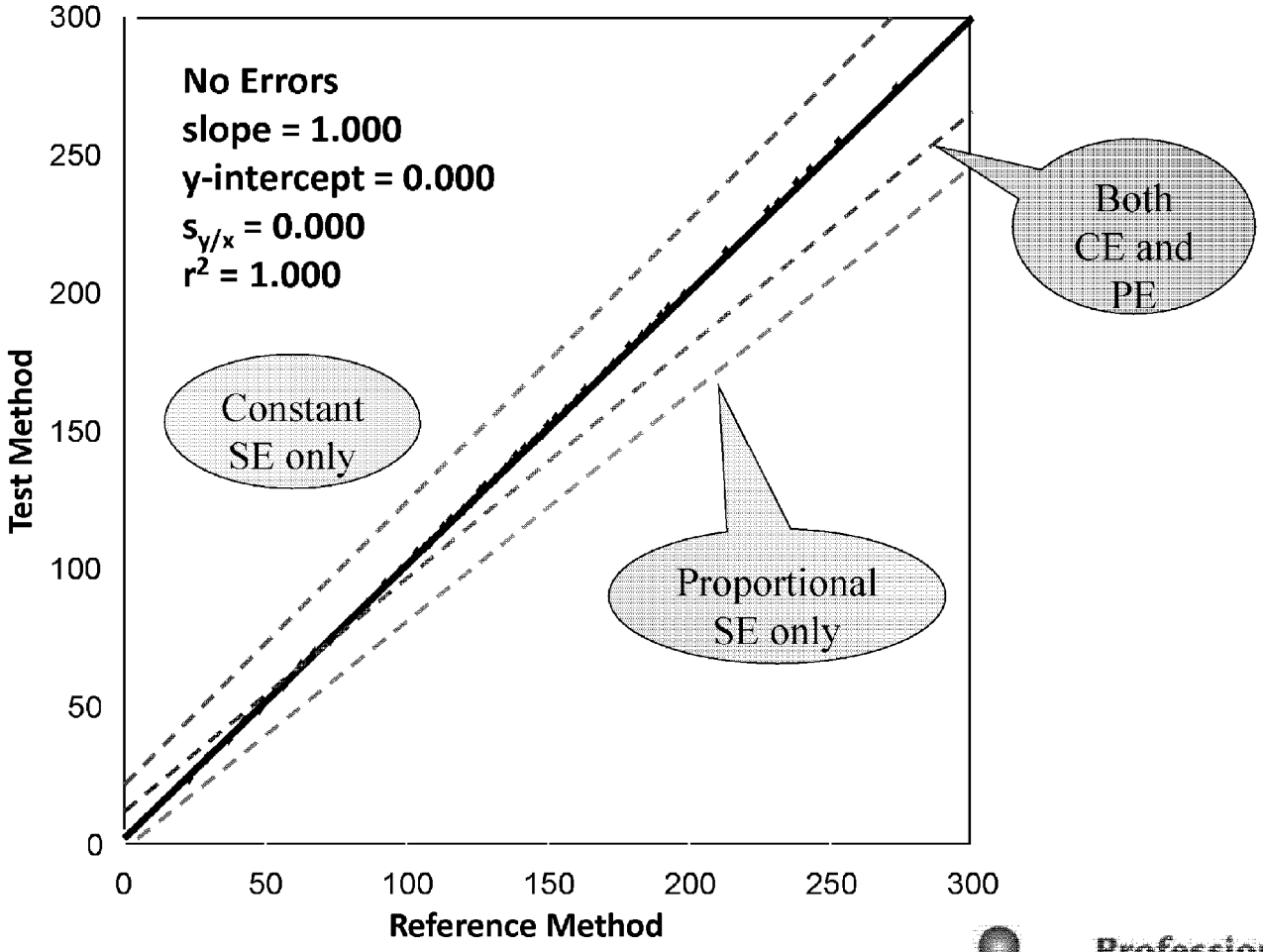




Professional Practice in Clinical Chemistry

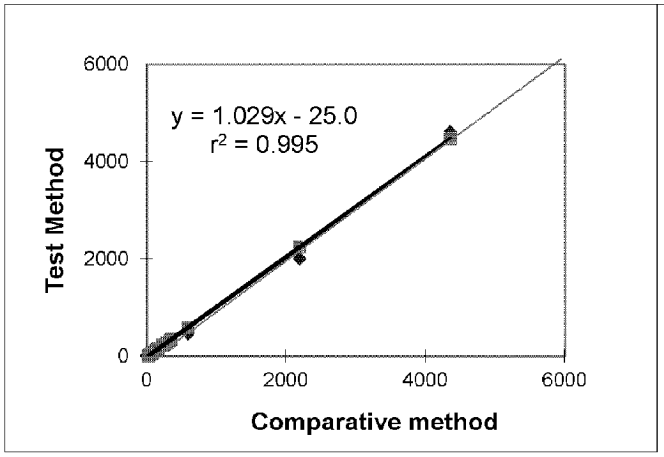
PFM-SEC v. Balwani-0007686

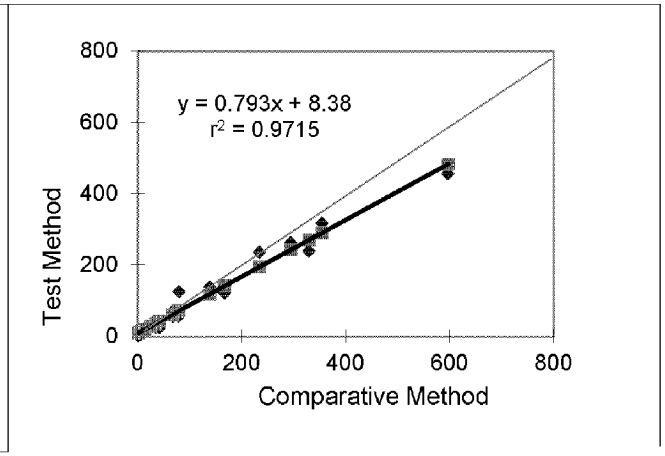
"Ideal" Regression Plot/Data

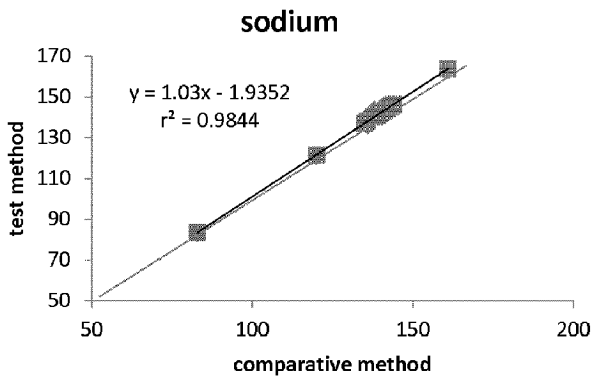


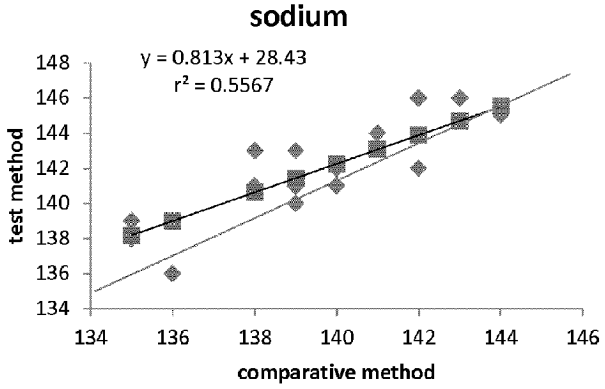
Professional Practice in Clinical Chemistry

Narrow and Unevenly Distributed Range of Analyte Concentrations – effect on Correlation Coefficient and Regression





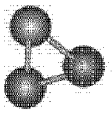




Professional Practice in Clinical Chemistry

Case1: Glucose: Total Error

- Method evaluation should also ensure that the magnitude of the errors affecting the results are acceptable
  - Evaluate the Total Error (TE) in the assay
    - TE = Random Error (RE) + Systemic Error (SE)
      - RE = SD_{TM} - largest component of TE
      - SE = Bias: determined by regression equation; plugging in medical decision point value
      - Marginal method 2SD < TE
      - Fair method 3SD < TE
      - Good method 4SD < TE
      - Outstanding method 6SD < TE



Professional Practice in Clinical Chemistry

Allowable Error (E_A)

"Acceptable" analytic error is decided using a **performance standard** (PS) based on the maximum allowable error (E_A) at a medical decision concentration of analyte (X_C).

Analyte	Acceptable Performance, ±	X_C	E_A	Max SD, CLIA	Max SD, Fraser
ALT	20%	50 U/L	10	2.5	5.8
Calcium	1.0 mg/dL	10.8 mg/dL	1.0	0.25	0.10
Glucose	10%	126 mg/dL	12.6	3.2	2.8
pH	0.04	7.35	0.04	0.01	0.01
		7.45	0.04	0.01	0.01
pCO_2	5 mm Hg	35 mm Hg	5.0	1.25	0.84

Example: Glucose PS = ±10% @ X_C

If X_C = 200 mg/dL

E_A = 20 mg/dL

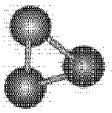
Case 1: Glucose: total error

- Evaluate TE of Test Method (TM)
  - RE of Glucose TM
  - From an inter-assay precision study, SD_{TM} @ [glucose] = 200 mg/dL was 3.5 mg/dL
  - RE = 4 SD_{TM} = 4(3.5) = 14 mg/dL

Professional Practice in Clinical Chemistry

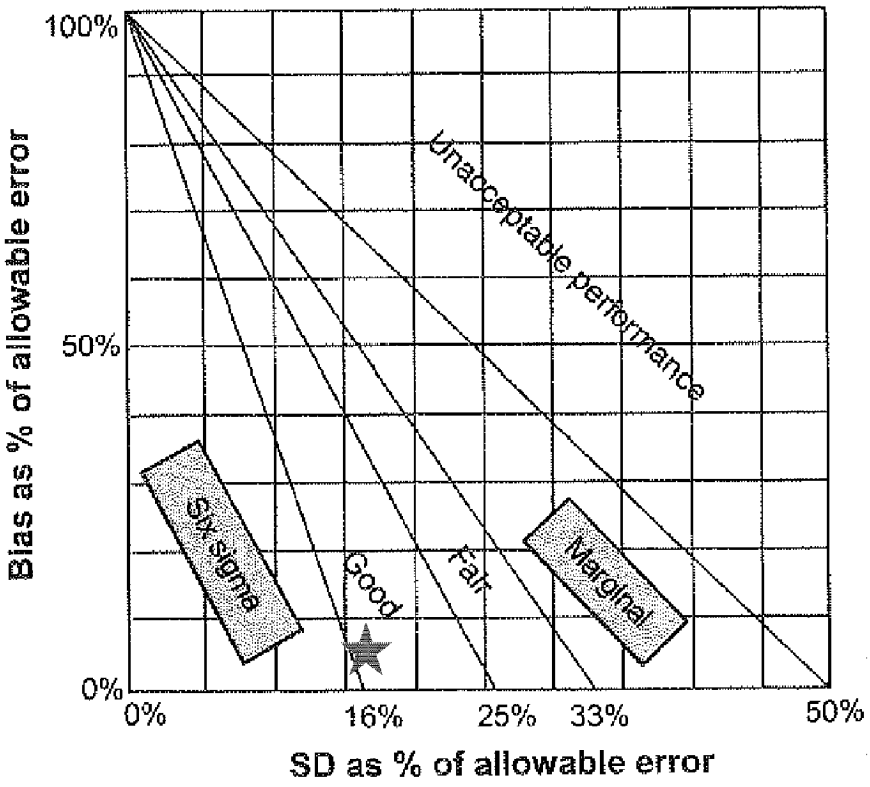
Case 1: Glucose: total error

- SE(bias) of Glucose TM
  - From linear regression equation for glucose
    - slope = 0.997, y-intercept = -0.34
    - $Y_C = 0.997X - 0.34$
  - Therefore, @ [glucose] = X_C = 200 mg/dL
  - $Y_C = 0.997(200)-0.34 = 199.06$
- $SE = |Y_C - X_C| = |200.00 - 199.06| = 0.94$ mg/dL
- TE = RE + SE = 14 + 0.94 = 14.94 mg/dL



Professional Practice in Clinical Chemistry

Case 1: glucose: method decision charts



Bias = 0.94 = 4.5%
SD = 3.5= 17.5%

Professional Practice in Clinical Chemistry

Case 2: The Bad: LDTs

- Modified FDA-approved, In-house, Scratch, Homebrew – Laboratory Developed Test
  1. **Accuracy**
  2. **Precision**
  3. **Reportable range**
  4. **Reference interval**
  5. **Analytical sensitivity – (lower limit)**
  6. **Analytical specificity – (interferences)**
  7. **Other specifications**

Professional Practice in Clinical Chemistry

Case 2: The Bad: LDTs

- Precision studies
- Analytical Measurement Range study

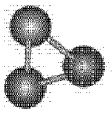
- Accuracy
  - Assay comparison/correlation study
  - Recovery
  - Analyte identity
- Lower limit of detection/quantitation
  - How low can you go
- Interferences Study
  - No manufacturer's data
- Reference Interval Study
  - How much should be there

- Clinical validity - sensitivity, specificity, predictive values

Professional Practice in Clinical Chemistry

3-Hydroxy-fatty acids – (3OHFA)

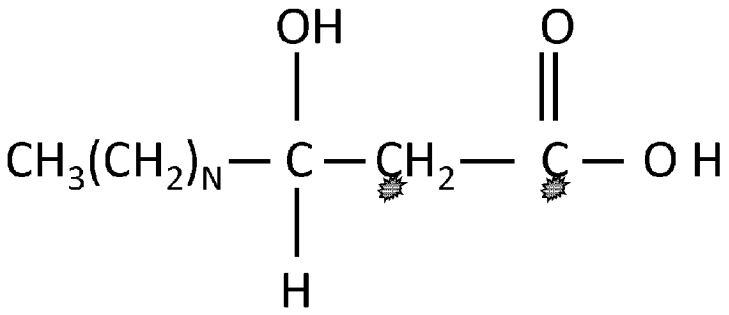
- Intermediates of Mitochondrial fatty acid oxidation
  - Serum build-up of 3-OHFAs indicates deficiencies of 3-hydroxy-acyl-CoA dehydrogenases
  - Diagnosis of LCHAD, SCHAD and TFP deficiencies
  - Stable-isotope dilution, electron impact ionization Gas Chromatography-Mass Spectrometry



Professional Practice in Clinical Chemistry

3-Hydroxy-fatty acids

- Background:
  - Assay measures 6 different chain length 3OHFAs
  - 6 different Isotope-labeled standards, 6C – 16C
  - C^{13} in place of C^{12} in two places in the compound
  - example:



Professional Practice in Clinical Chemistry

Case 2: 3OHFA: reportable range

- Analytical measurement range
  - tried 0.001 - 500 μM
  - best range 0.2 - 50 μM (range of linearity)
  - LOQ 0.2μM

- Concentration of isotope-labeled standards
  - Internal Standard ≅ Analyte concentration
  - tried 0.1 - 100 μM 10μM

Professional Practice in Clinical Chemistry

Case 2: 3OHFAs: analytical sensitivity: lower limit

- Limit of detection (limit of absence)
  - Assay patient sample with no measurable analyte present multiple (20 times) – LOD = mean ± 3SD
  - Assay zero calibrator
  - 3OHFA - 0.05 calibrator
    - Mean + 3SD = 0.0195 + 0.078 = 0.0975 = 0.1 μM

- Limit of quantitation (functional sensitivity)
  - Minimum concentration that can be reproducibly measured at an acceptable CV – CV based on biological variation, i.e. CV < 20% TSH
  - CV at 0.2 μM = 5 – 10%

Professional Practice in Clinical Chemistry

Case 2: 3OHFAs: precision

- Precision
  - Within-assay (20 points)
    - low (0.3 μM): cv 4 - 8 %
    - high (30 μM): cv 1 - 4 %
  - Between-assay (25 points)
    - low (0.3 μM): cv 4 - 15 %
    - high (30 μM): cv 1 - 2 %

Professional Practice in Clinical Chemistry

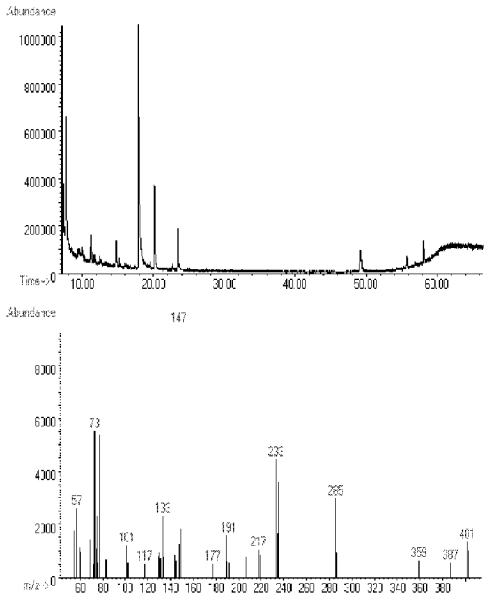
Case 2: 3OHFAs: Accuracy

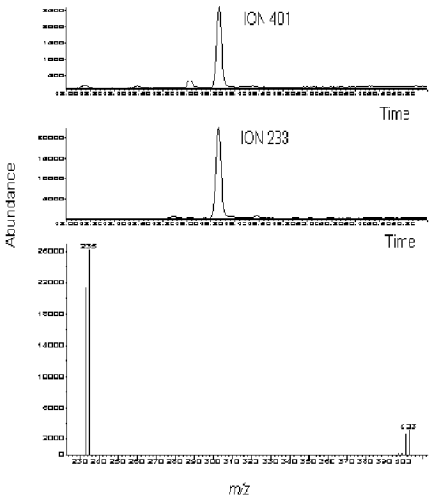
- Correlation?
- Analyte Identity
- Recovery

Professional Practice in Clinical Chemistry

Case 2: 3OHFAs – analyte identity

- Analyte Identity - analyzed each chain length native and isotopic compound via GC/MS to determine fragmentation pattern and appropriate ions to use for identification and quantitation



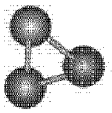


Professional Practice in Clinical Chemistry

Case 2: 3OHFAs: analyte identity

– Stable isotope standards

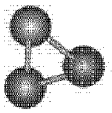
3-OHFA	Native MW Quant	Isotope MW Quant	Native MW confirm	Isotope MW confirm	Retention time (min)
3-OH-C6	261	263	233	235	16 – 19
3-OH-C8	289	291	233	235	22 – 25
3-OH-C10	317	319	233	235	28 – 32
3-OH-C12	345	347	233	235	36 – 39
3-OH-C14	373	375	233	235	43 – 47
3-OH-C16	401	403	233	235	48 – 51



Professional Practice in Clinical Chemistry

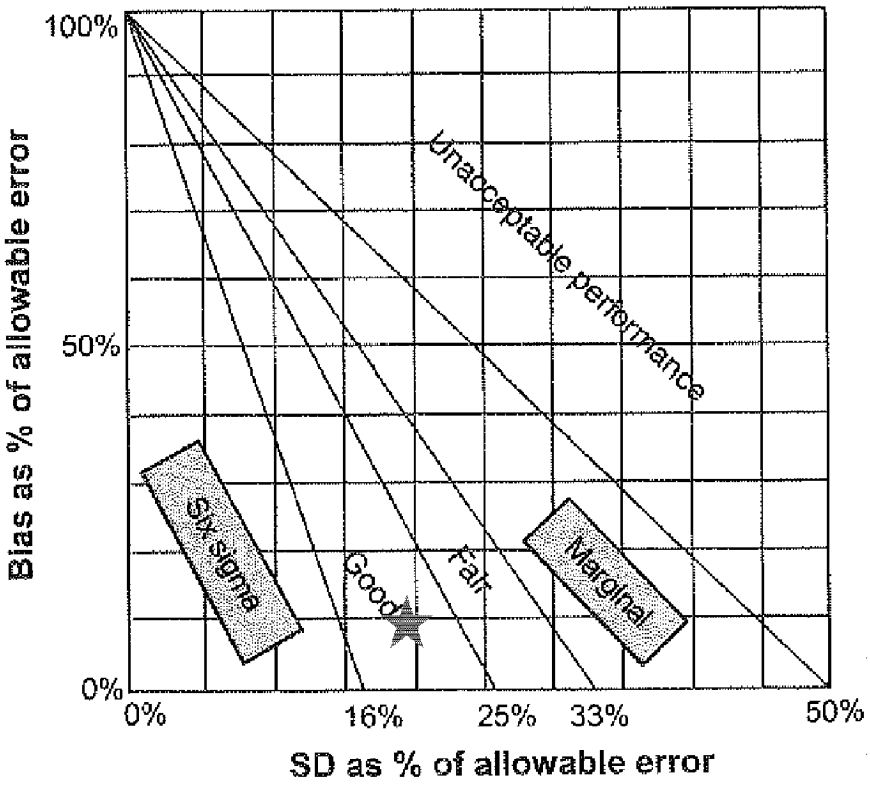
Case 2: 3OHFAs: recovery

- Accuracy - recovery
  - Target — % recovery
    - (0.3 μM): 87 - 103
    - (8.0 μM): 89 - 108
    - (30 μM): 94 - 100



Professional Practice in Clinical Chemistry

Case 2: 3-OHFA: method decision charts

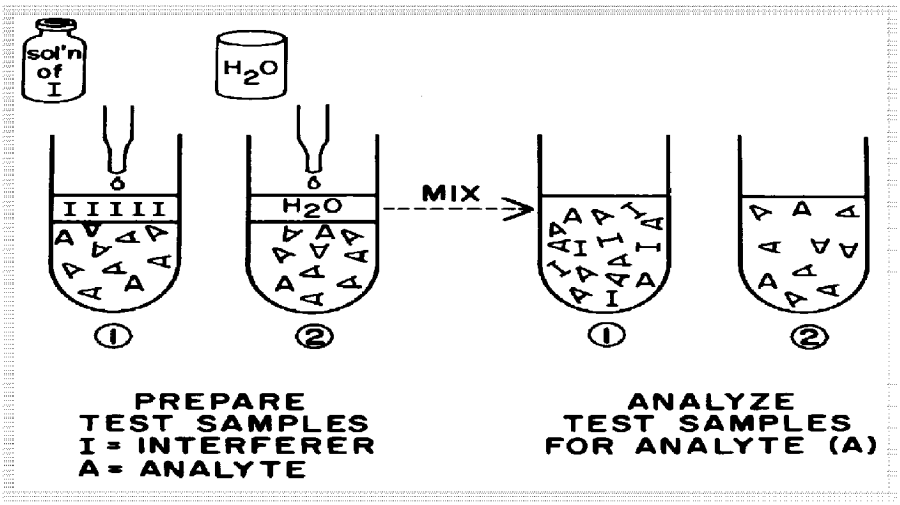


Total Error = 0.5

Bias = 0.04 = 8%
SD = 0.1= 20%

Professional Practice in Clinical Chemistry

Case 2: 3OHFAs: specificity (interferences)



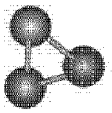
Interference Experiment Notes:

1. Volume of interferent should be ≤10% of total volume
2. Use several samples per interferent
3. At a minimum, test samples in duplicate

Professional Practice in Clinical Chemistry

3-Hydroxy-fatty acids

- Interferences
  - No:
    - Hgb, Bilirubin, Lipemia
    - Spike with badly hemolyzed sample
      - Get same result – hemolysis does not affect assay
      - Get a different result – begin spiking with increasingly dilute hemolyzed sample
  - Yes:
    - Citrate large 347 ion in citrate



Professional Practice in Clinical Chemistry

Case 2: 3-OHFAs: reference interval

- Reference Interval Study
  - **ESTABLISH** an Interval, rather than validate one
  - Typically, require a minimum of 120 specimens from "healthy" individuals
  - Specimens from "healthy" individuals – medically well; taking no drugs, herbals, vitamins, or other substances likely to affect analyte values
  - Pediatrics!

Professional Practice in Clinical Chemistry

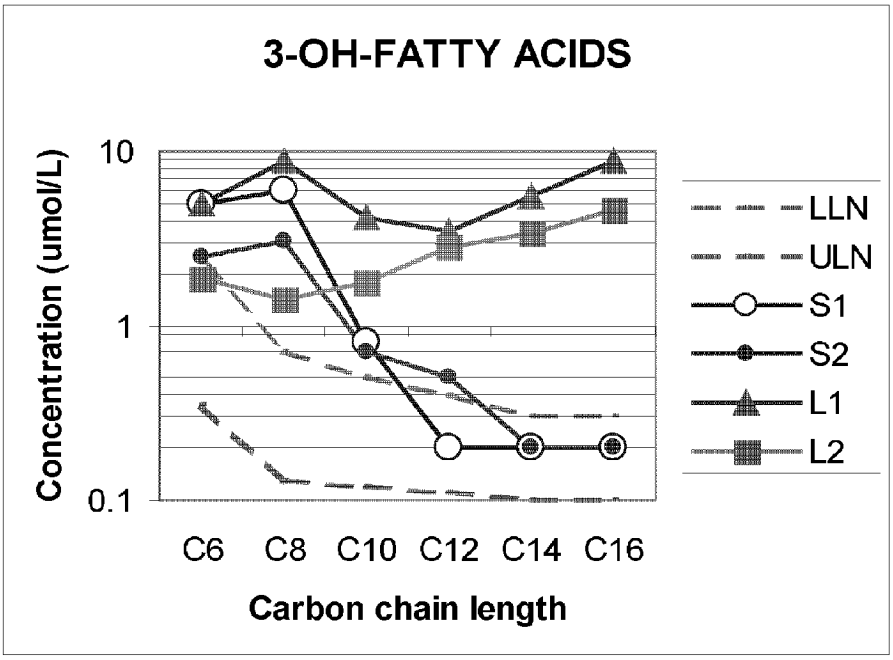
Case 2: 3OHFAs: reference intervals

- Reference intervals

3-OH-FA	normal range (µmol/L)	MCT range (µmol/L)
3-OH-C6	0.4 - 2.3	1.6 - 18.9
3-OH-C8	0.2 – 1.0	0.4 - 8.3
3-OH-C10	0.2 - 0.6	0.2 - 2.3
3-OH-C12	0.2 - 0.6	0.2 - 1.4
3-OH-C14	< 0.4	< 0.9
3-OH-C16	< 0.5	< 0.8

Professional Practice in Clinical Chemistry

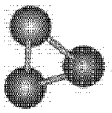
3-Hydroxy-fatty acids



Professional Practice in Clinical Chemistry

Case 2: 3OHFAs

- Clinical Validity
  - Sensitivity
    - For LCHAD = 100%
    - For SCHAD = 100%
  - Specificity
    - For LCHAD = 100%
    - For SCHAD = 89.8%
  - Positive predictive value
    - For LCHAD = 100%
    - For SCHAD = 26.8%
  - Negative predictive value
    - For LCHAD = 100%
    - For SCHAD = 100%



Professional Practice in Clinical Chemistry

Case 2: 3OHFAs

children's MEDICAL CENTER OF DALLAS	SECTION: GENERAL/CHEMISTRY SUBJECT: NEW METHOD / INSTRUMENT CHECKLIST
Prepared by: Patti Jones Edited by: Patti Jones	Supersedes: new Revised date: 05/01/2003

INSTRUMENT GC/MS TEST 3-OH-fatty acids

Final signoff

VALIDATION	Accept	Comments	Signature	Date
Linearity (analytical measurement range)	✓	see paper	Flores	2/2000
Precision (Reproducibility)	✓	"	Flores	2/2000
Sensitivity (Lower limit of detection)	✓	"	Flores	2/2000
Accuracy (Comparison / Correlation)	✓	"	Flores	2/2000
Reference range (normal patients)	✓	"	Flores	2/2000
Specificity (Interferences)	✓	"	Flores	2/2000

New method / instrument is acceptable and put in use on 2/1/2000 (date).

Technologist Flores date 2/1/2000

Clinical Consultant Flores date 2/1/2000

•**Jones PM**, Quinn R, Fennessey PV, Tjoa S, Goodman SI, Fiore S, Burlina AB, Rinaldo P, Boriack, RL, Bennett MJ. Improved stable isotope dilution-gas chromatography-mass spectrometry method for serum or plasma free 3-hydroxy-fatty acids and its utility for the study of disorders of mitochondrial fatty acid β-oxidation. Clin Chem. 2000 46(2):149-155.

Professional Practice in Clinical Chemistry

Case 3: the molecular: CMV by real time PCR

- Start out by setting up assay and optimizing
  - Selection of primers
  - Tweaking master mix
  - Optimizing temperatures and times in each cycle

  After optimization – validation!

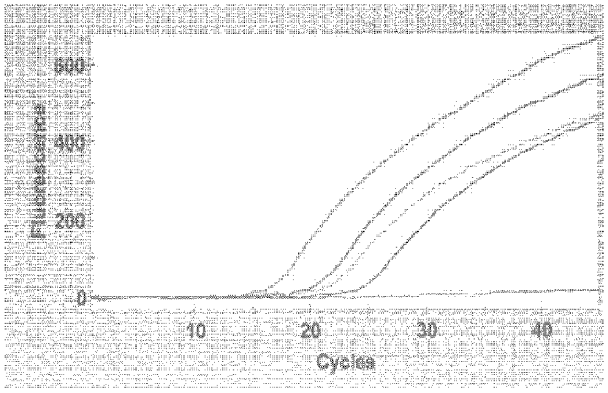
Professional Practice in Clinical Chemistry

Case 3: CMV by real time PCR

- Follow CLIA guidelines - LDTs
  1. Accuracy
  2. Precision
  3. Reportable range
  4. Reference interval
  5. Analytical sensitivity
  6. Analytical specificity
  7. Other specifications

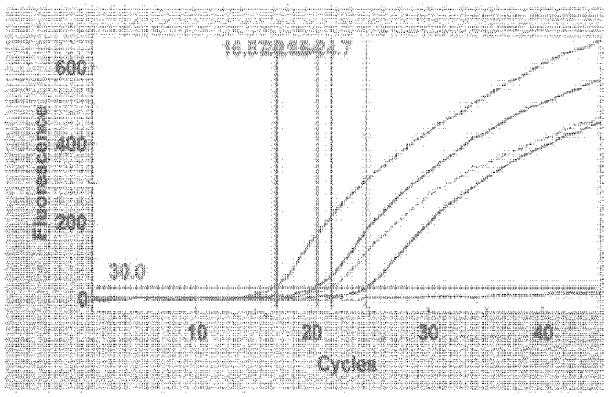
Professional Practice in Clinical Chemistry

Case 3: CMV by real time PCR



- Reportable range
  - AMR/linearity

standard curve - day 7: TET (Mar 12, 2001 10:25 AM)

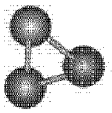


- Testing 10^2 copies per mL to 10^5 copies per mL – determining cycle time (CT) equivalent for viral DNA copies

Professional Practice in Clinical Chemistry

Case 3: CMV by real time PCR

- Reportable range
  - 1000 copies/mL to 5,000,000 copies/mL.
  - Linear in this range of quantitation with a correlation coefficient of 0.998. (graphing CT vs viral copies)
- Precision
  - **Within-assay variation:** Ranged from 0.5% to 4.3% by CT value, and from 11% to 56% by viral copy number
  - **Between-assay variation:** Ranged from 1.7% to 3.9% by CT value, and from 23% to 70% by viral copy number



Professional Practice in Clinical Chemistry

Case 3: CMV by real time PCR

- Accuracy
- 20 paired whole blood samples encompassing the linear range were assayed at CMC and Focus Dx. Correlation coefficient between the two assays was 0.93.

Professional Practice in Clinical Chemistry

Case 3: CMV by real time PCR

- Analytical sensitivity – lower limit
  - **Limit of Quantitation:** 1000 copies/mL
- Analytical specificity – interferences
  - No cross-reactivity detected with other Herpes viruses or organisms common in transplant recipients.
- Reference interval –
  - Undetectable

Professional Practice in Clinical Chemistry

Case 4: Glutaric Acidemia, type 1 (GA1)-GCDH gene sequencing

- Extraction of DNA from fibroblast cell culture
- Amplification of DNA via RT-PCR
- Purification of PCR product DNA
- Sequencing of DNA
- Reviewing data and identification of gene sequence mutations

Professional Practice in Clinical Chemistry

Case 4: Glutaric Acidemia, type 1 (GA1)-GCDH gene sequencing

- Follow CLIA guidelines - LDTs
  - Accuracy
    - 10 fibroblast cultures purchased from Coriell Institute for Medical Research, each harboring a specific change in the GCDH gene. The assay detected all changes in 100% of the cell lines, and also detected some changes that were not specified by Coriell.
  - Precision
    - Same samples assayed repeatedly – same results
  - Reportable range
    - Can detect Heterozygous wild type, heterozygous mutant, homozygous wild type, homozygous mutant

Professional Practice in Clinical Chemistry

Case 4: Glutaric Acidemia, type 1 (GA1)-GCDH gene sequencing

- Follow CLIA guidelines – LDTs
  - Analytical sensitivity
    - **Limit of Detection (LOD):** 50 ng of DNA per PCR reaction
  - Analytical specificity
    - No cross-reactivity with other regions of the genome was detected. PCR products produced single amplification bands of the expected sizes. Sequencing primers used in this assay were confirmed to be complementary to regions of the GCDH gene.
  - Reference interval
    - No mutations detected – homozygous wild type

Professional Practice in Clinical Chemistry

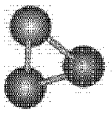
Case 4: Glutaric Acidemia, type 1 (GA1)-GCDH gene sequencing

- Follow CLIA guidelines – LDTs
  - Other specifications
    - **Clinical Validity:**
      - GA-1 is an autosomal recessive disorder of lysine, hydroxylysine, and tryptophan metabolism caused by deficiency of glutaryl-CoA dehydrogenase enzyme.
      - Numerous publications have correlated GCDH mutations with the onset of Glutaric Acidemia type I.
      - Recognition of this disorder before onset of neurological symptoms is essential for treatment and prevention of permanent damage.
      - Standard acylcarnitine and organic acid assays may miss this disorder in the case of "low-excretors" .

Professional Practice in Clinical Chemistry

Self Assessment Questions

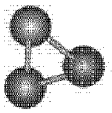
1. FDA-approved tests require validation of the following parameters:
   a) Precision, accuracy, interferences, lower limit of quantitation
   b) Precision, interferences, reference interval, lower limit of quantitation
   c) AMR, precision, accuracy, lower limit
   d) Precision, AMR, reference interval, accuracy



Professional Practice in Clinical Chemistry

Self Assessment Questions

2. LDTs:
   a) Should be validated for total error and for clinical validity before use
   b) Require validation of precision, accuracy, AMR and reference intervals only
   c) Do not need validation of interferences or lower limit of quantitation
   d) Do not include modified FDA-approved tests



Professional Practice in Clinical Chemistry

Self Assessment Questions

3. Molecular tests
   a) Do not require CLIA validation before clinical use
   b) Only require validation of precision, accuracy, AMR, reference interval
   c) Require the same criteria be validated as any LDT
   d) Cannot be offered by a CLIA certified lab

Professional Practice in Clinical Chemistry

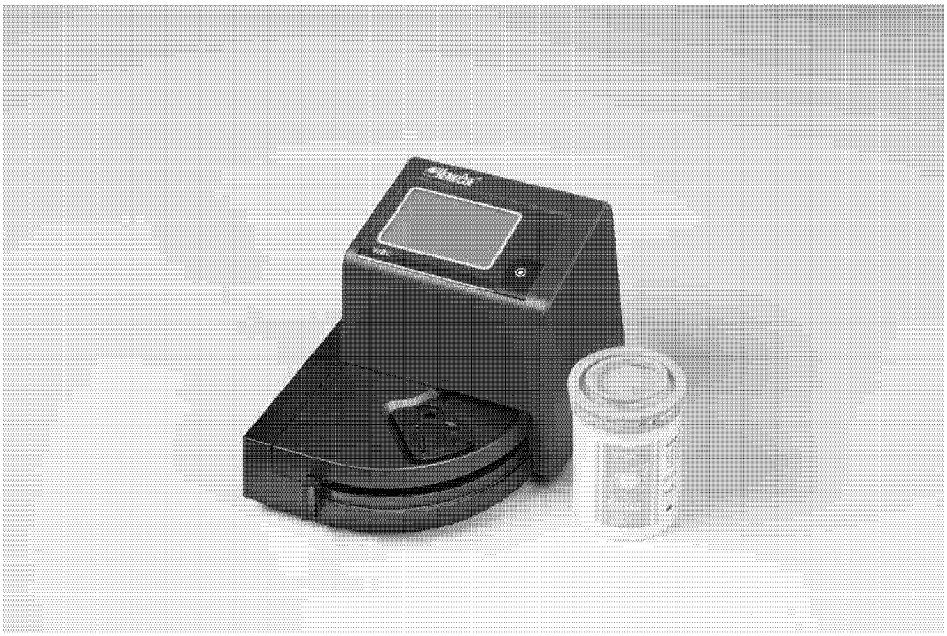
HEMOCUE®
A Quest Diagnostics Company

CLIA Waiver Application

CLIA Categorization Only
Previously Cleared Device

HemoCue WBC System

(K071652)



Official Correspondent:
Allan White
Quality Systems Manager
HemoCue, Inc.
40 Empire Drive
Lake Forest, CA 92630-2244
(800) 881-1611 x110
(949) 859-3066 FAX
allan@hemocue.com

On behalf of:
HemoCue AB
Kuvettgatan 1
S-262 71 Angelholm,
SWEDEN

Table of Contents

1 Introduction

A point of care test for total white blood cell count (WBC) has been developed by HemoCue AB. The test is simple and can be performed in any doctor's office. The test is performed using a small amount of capillary or venous whole blood and the answer is available within minutes. The HemoCue WBC system is used for a quantitative determination of the white blood cell count.

The purpose of this submission is to demonstrate that HemoCue WBC System is simple to use and accurate in the hands of the intended operators and that there is an insignificant risk to obtain an erroneous result.

2 Description of the HemoCue WBC System

2.1 Indications For Use

The HemoCue WBC System is indicated for use for quantitative determination of white blood cell (WBC) count in capillary or venous whole blood. The HemoCue WBC System is for *In Vitro* Diagnostic use only. The HemoCue WBC Analyzer is only to be used with HemoCue WBC Microcuvettes. The HemoCue WBC system is indicated for use in clinical laboratories and for point-of-care settings.

2.2 The Measuring Principle

The system consists of the HemoCue WBC Analyzer together with specially designed microcuvettes, the HemoCue WBC Microcuvettes. The microcuvette serves both as a sample container and a reaction chamber. The microcuvette is for single-use only. A blood sample of approximately 10 µL is drawn into the cavity by capillary action. A hemolysing agent lyses the red blood cells in the microcuvette and a staining agent colors the white blood cells. An image is taken of the stained white blood cells and the number of cells is counted by image analysis. The result is presented within 3 minutes on the analyzer's display.

2.3 Physical description of the system

2.3.1 The HemoCue WBC Analyzer

The HemoCue WBC Analyzer (Figure 2.1) is a portable device. The main parts are the cuvette holder (in which the microcuvette is placed), the cuvette moving arm (brings the microcuvette into correct measuring position), a magnifying optic unit, a camera, image processing software, a display and a power adapter.

Figure 2.1. The HemoCue WBC Analyzer

Technical Specifications

Optional output	RS232 for printer connection
Power	Batteries: 6 batteries type AA Power adapter: CE marked, UL approved Input: 100-240 VAC/50-60 Hz/200mA Only use adapters recommended by HemoCue, as listed under "Approved Adapters" in the Operating Manual
Physical characteristics	Dimensions: 185x133x120 mm (7.28 x 5.24 x 4.72 inches) Weight: 600g (1.32 pounds) (with batteries installed)
Operating environment	15-35 ºC (59-95 ºF), < 90 % non-condensing humidity. Allow the analyzer to reach ambient temperature before use.
Storage environment	0-50 ºC (32-122 ºF), < 90 % non-condensing humidity

2.3.2 The HemoCue WBC Microcuvette

The HemoCue WBC Microcuvette (Figure 2.2) is made of polystyrene plastic and contains saponin that hemolyzes the red blood cells, methylene blue that stains the white blood cells and nonactive reagents. A blood sample of approximately 10 µL is drawn into the cavity by capillary action. The microcuvette serves as sample container and a reaction chamber. No dilution of the sample is required.

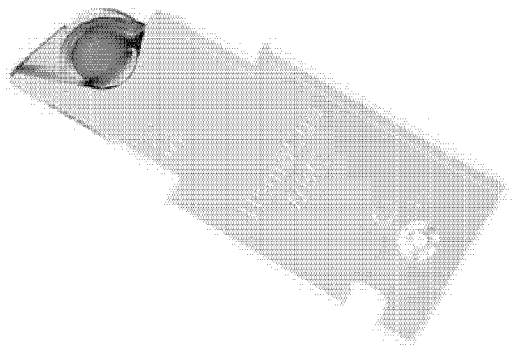


Figure 2.2. The HemoCue WBC Microcuvette

Technical Specifications

Composition	The microcuvette is made of polystyrene plastic and contains the following reagent: <20 µg/g microcuvette methylene blue, <500 µg/g microcuvette saponin, <400 µg/g microcuvette Surfynol 465, <400 µg/g microcuvette Triton X-100.
Physical characteristics	Vials with 40 microcuvettes in each vial

Storage conditions	15-35 °C (59–95 °F), < 90 % non-condensing humidity. An unopened vial of microcuvettes can be stored for a shorter period of time (up to 4 weeks) outside specified storage conditions (down to 0°C (32°F) and up to 50°C (122°F)

2.4 Accessories and Spare Parts

The following accessories and spare parts are available:

Spare parts

Power adapter

Cuvette Holder

Accessories

HemoCue Cleaner WBC

Optional accessories but not supplied

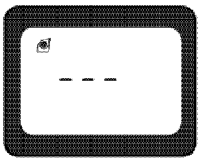
Lancets

2.5 Description of an Analysis

2.5.1 Test Procedure

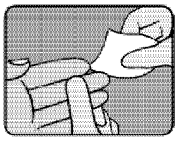
Please note that the complete test procedure should be read before performing the test.

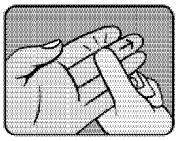
1. Pull the cuvette moving arm out to the loading position. Press and hold the button until the display is activated. The three flashing dashes and the HemoCue symbol indicates that the analyzer is ready for use.



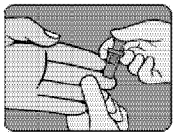
2. Take a HemoCue WBC Microcuvette from the vial. Keep unused cuvettes in the original package. **Do not remeasure old cuvettes.**

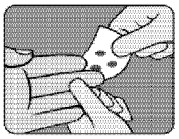
3. Make sure the patient's hand is warm and relaxed. Use only the middle or ring finger for sampling. Avoid fingers with rings on. Clean fingertip with disinfectant and allow to dry completely or wipe off with a dry, lint-free wipe.
Using your thumb, lightly press the finger from the top of the knuckle towards the tip.



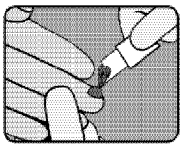


4. While applying light pressure towards the fingertip, puncture the finger using a lancet.
Wipe away the first two or three drops of blood.

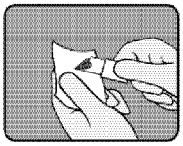




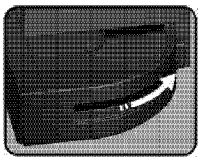
5. When the blood drop is large enough, fill the microcuvette in one continuous process. **Do NOT refill the cuvette!** NOTE: Make sure that the microcuvette is filled from the tip, placed at about a 45 degree angle towards the blood drop. Look for air bubbles in the filled microcuvette. If present, discard the microcuvette and fill a new microcuvette from a new drop of blood. Small bubbles around the edge can be ignored.



6. Wipe off excess blood from the outside of the microcuvette with a clean, lint-free wipe. Do not touch the open end of the microcuvette.

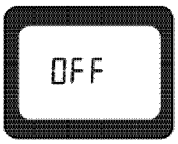


7. Place the filled microcuvette in the cuvette holder within 40 seconds after filling. Gently push the cuvette moving arm towards the measuring position. It will automatically slide to the measuring position and the measurement starts.



8. After approximately 3 minutes, the WBC value is displayed as the number of WBC/L in the specimen. The result will remain on the display as long as the cuvette moving arm is in the measuring position. Do not remeasure the filled microcuvette.
Note! Always handle blood specimens with care, as they might be infectious. Always wear protective gloves when handling blood specimens. Consult local environmental authorities for proper disposal.

9. To turn the analyzer off, press and hold the button until the display reads OFF and then goes blank.



2.5.2 Results

The display shows a numerical value which is the number of white blood cells in the specimen within the measuring range 0.3-30.0 x 10^9/L.

"HHH"	Measurements above 30.0 x10^9/L are displayed as "HHH".
"LLL"	Measurements below 0.3 x10^9/L are displayed as "LLL".
"ErrXX"	"ErrXX" means that an error has occurred. See the Troubleshooting Guide in the operating manual for additional information.

3 Demonstrating that HemoCue WBC System is Simple to Use

HemoCue WBC system is a fully automated instrument not requiring any intervention by the operator during the analysis. The system uses direct unprocessed specimen not requiring any manipulation of the specimen or of the reagent by the user.

The operation of HemoCue WBC system does not require any special training of the operator. The HemoCue WBC Analyzer only has one button, the on/off button, which makes the instrument/user interface very simple. The operating manual includes a thorough troubleshooting guide with interpretation of the error codes with explanation and actions that should be taken by the user. A Quick Reference Guide as well as a training video on CD-ROM are provided to the users at delivery of the device. Performance studies have been conducted to demonstrate that the results obtained by the intended user are comparable with results obtained by a professional user and a reference method.

The system does not require any electronic or mechanical maintenance. If an error code is shown, the troubleshooting guide in the operating manual clearly indicates if the analyzer needs to be sent to HemoCue for technical support.

The HemoCue WBC system performs a system integrity test for every single measurement. A set of checks are performed at startup and in between the measurements. When passing the self test, the display will show the HemoCue symbol and three flashing dashes, indicating that the analyzer is ready to perform a measurement. An error code will be displayed if the self test fails.

A blanking test is performed prior to the first measurement after start-up of the analyzer and when the cuvette moving arm is moved to its outer position during normal operation.
A system suitability test is performed for each measurement by utilizing the focal quality of the stained cells. If the cells cannot be focused properly, an error code will appear and the measurement is interrupted.
Several internal checks ensure that only areas with suitable cell distribution are used for image analysis. No additional quality controls are required for verification of the system functionality.

Critical error codes locks the ordinary program flow until the analyzer is turned off. When a non-critical error occurs, the analyzer is reset after blanking.
Definition: Non critical errors are errors that are corrected by rejecting the measurement.

HemoCue has not identified any failure modes where use of a quality control material would provide additional assurance of the system's performance than the built-in self test contained in the HemoCue WBC Analyzer.

When an analysis has been performed with the HemoCue WBC system, the results given are shown in numerical figures, not requiring the user to further interpret or conduct subsequent calculations.

Based on the above information, HemoCue AB considers the HemoCue WBC system simple to use.

4 Demonstrating Insignificant Risk of an Erroneous Result – Failure Alert and Fail-Safe Mechanisms

4.1 Risk Analysis

A risk analysis for the HemoCue WBC system for risks associated with hazards and hazardous situations due to user skills, human factors and foreseeable misuse has been performed.

The risk management has been performed according to ISO 14971 and HemoCue's internal procedure for risk management (QI-018). See Attachment 1 for the risk analysis.

4.2 Failure Alert and Fail-Safe Mechanisms

Based on the results of the risk analysis, flex studies have been conducted. The flex studies have been designed to challenge the system under conditions of stress to identify potential user related failures and determine the robustness of the system in the hands of the user. Studies regarding fail-safe and failure mechanisms have been conducted under conditions that stress the device in order to demonstrate how fail-safe and failure alert mechanism respond to such conditions.

Table 4.1.

Potential source of error identified (Risk analysis ID# Attachment 1)	Type of study	Mean of bias (%) with 95 % confidence level or error codes	Acceptance criteria	Comments on results
Angle of filling. Deviation from the defined procedure (filling angle 45°and a filling place at the edge of the microcuvette) Risk No. 19	Flex study consist of filling angles and filling places: Filling angle 0° Filling angle 90° Filling angle -90° Filling place No 1 Filling place No 2	 -0.4 to 1.7 -0.8 to 1.0 0.8 to 3.0 -0.7 to 1.6 0.2 to 2.5	-15 to +15 or error code	All filling angles as filling places are approved
Time of latency. Deviation from the defined procedure (< 40 s) Risk No. 32 and 33	Flex study consist of different time of latency: 1 minute 3 minutes 5 minutes 10 minutes	 -0.7 to 1.8 -0.1 to 2.3 0.1 to 3.2 -1.3 to 2.0	-15 to +15 or error code	All time of latency are approved
Try to run an analysis without filling the microcuvette with sample (an unfilled	Study to show that empty cuvette will give an error code	Error code	Error code	Approved

microcuvette placed in measuring position) Risk No. 25				
Partly filled microcuvette (the microcuvette should be completely filled (10µL) in one step) Risk No. 15, 16 and 24	Flex study consist of different level of filled cuvette: filled with 5 µL sample filled with 2 µL sample Empty cuvette	 -1.9 to +0.2 Error code Error code	-15 to +15 or error code	Approved results
Sample dilution. (undiluted sample should be used) Not identified as a foreseeable risk. However, flex study has been done.	Flex study consist of different dilutions: Dilution factor 1:5 Dilution factor 1:10 Dilution factor 1:20	 -8.5 to +8.9 -3.7 to +7.6 -8.8 to +10.5	-15 to +15	All dilution factors approved
Storage of sample outside defined temperature conditions. (Defined conditions are storage in room temperature (r.t.) Up to 48 hours stability in r.t.) Risk No 30, 31 and 38	Study consist of two temperatures: 48 hours in refrigerator 48 hours in room temperature	 +1.9 to +11.6 -3.3 to +1.2	-15 to +15	All tested temperatures approved
Operating temperature outside defined conditions. (Defined conditions is room temperature (r.t.) for both cuvette and sample) Risk No. 1 and 2	Flex study consist of different cuvette and sample temperature: r.t./ +7°C r.t./+37°C +7°C /+r.t. +7°C./+37°C	 -3.8 to -1.4 -1.2 to +1.0 -0.6 to +1.3 -1.4 to +0.1	-15 to +15 or error code	All tested temperatures for sample and cuvette approved

The microcuvette is filled in two steps. (the microcuvette should be filled in one step) Risk No. 20	Flex study consist of filling in two steps: 5 + 10 µL 2 + 13 µL	 -11.8 to -8.2 0.2 to 2.7	-15 to +15 or error code	Both combinations approved *!Note in package insert*
Wrong type of sample. (capillary or venous sample can be analyzed) Risk No. 13 and 14	Flex study consist of different type of samples Human urine Human urine with blood Plasma	All samples not containing WBC below the measuring range (LLL)	LLL or error code	All types of samples approved
Wrong HemoCue product used, another type of microcuvette to another HemoCue system (the WBC microcuvette should be used) Risk No. 37	Flex study consist of different kind of microcuvettes from HemoCue systems Microcuvetts from Albumin 201, Monitor, Glucose 201 RT, B-Glucose, Glucose 201, B-Hb, Hb 201+, Hb 301, Plasma Low Hb, Donor Hb Checker	It was not possible to position any type of cuvette	Not possible to use any other cuvettes	All tested variants approved
The microcuvette is contaminated (e.g)/not clean Risk No. 21 and 22	Flex study consist of different kind of damaged cuvettes: - Scratched cuvettes on both sides - Cuvette with powder - Cuvette with finger prints	 -3.4 to -1.1 -1.1 to +1.1 -1.8 to 0.1	-15 to +15 or error code	All kind of damaged cuvettes gave approved results
Coagulation. Risk No. 28	Flex study consist of partly coagulated blood samples	Not possible to fill cuvettes with partly coagulated blood samples	-15 to +15 or error code	Approved results
93 % Humidity. (defined storage and	Verification study	-4.71 to -0.1	-15 to +15 or error code	Approved results

operating conditions < 90 % humidity) Risk No. 3				
Operating temperature (defined operating temperature 15 to 35°C) Risk No. 1 and 2	Flex study consist of different temperatures: +12°C +15°C +40°C	 +0.2 to +6.1 -2.4 to 1.9 -12.2 to -3.8	-15 to +15 or error code	All temperatures resulted in approved results
The microcuvette is contaminated with blood on the outer walls (the outside) due to poor sampling technique Risk No. 17 and 23	Verification study	error code E001	Error code	Approved results
High levels of WBC. (The measuring range is defined to 0.3 to 30 $x10^9$/L) Risk No. 5	Flex study consist of samples with leukocytes > 30 $x10^9$/L	≤ 100 HHH > 100 error code or HHH	Error code or HHH	Approved results

4.3 Conclusion

All results from conducted tests meet the pre-defined acceptance criteria. There is no significant risk of obtaining an erroneous result with the HemoCue WBC system when used by the intended user.

5 Demonstrating Insignificant Risk of an Erroneous Result – Accuracy

5.1 Objective with Field Study

The field study will demonstrate, as close as possible, how the HemoCue WBC System performs on actual clinical specimens by intended operators under the conditions of intended use.

5.2 Summary of Field Study

A comparison of the HemoCue WBC system used by an untrained intended user (WM) with the Sysmex XS-1000*i* used by a professional user (CM) was conducted. The study show that 96.6% of all results are within the ATE-zone.

No samples at the comparisons were found outside the Limits of Erroneous Results (LER), see below.

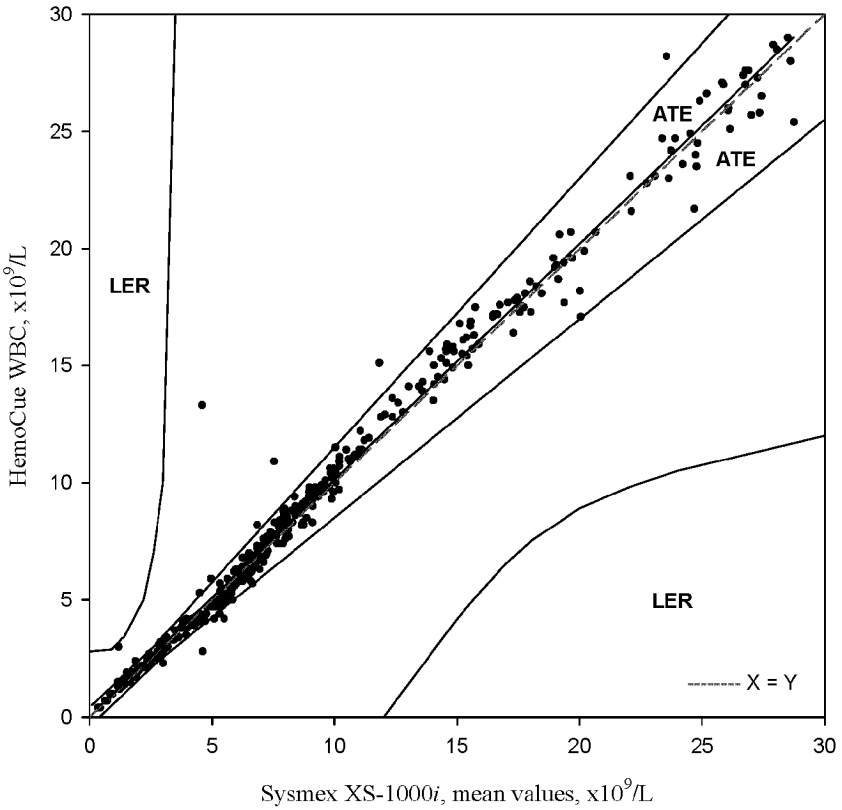


Figure 5.1. Scatter plot in Error grid, HemoCue WBC vs Sysmex XS-1000*i*, x10^9/L

The results in this study fulfil the acceptance criteria and hereby show that the HemoCue WBC System is appropriate for use in a CLIA waived laboratory according to the requirements in the CLIA Draft Guidance of 2005.

5.3 Design of Field Study

The field study was designed in order to comply with "The Draft Guidance for Industry and FDA Staff: Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications; Draft Guidance issued September 7, 2005".

5.4 Performance of Field Study

5.4.1 Intended Use Settings and Intended Users

Five intended use settings and a total of 11 intended users participated in the field study for the HemoCue WBC System.

Table 5.1. Summary of sites

Site	Number of capillary samples	Number of Venous samples	Total number of samples
Delfinen Primary Care	56 (2*)	70	126 (2*)
Munka Primary Care	74 (2*)	80	154 (2*)
Sjöcrona Primary Care	-	70	70
PTC at Ängelholm Hospital	-	60	60
Närlunda Primary Care	5	-	5
Total	135 (4*)	280	415 (4*)

*Number of samples excluded from calculations due to insufficient sample volume. or result outside defined measuring range, see section 5.4.7.2.

Delfinen, Munka, Sjöcrona and Närlunda Primary Care Centers are district health centers in primary care. They are equivalent to CLIA waived laboratories in the USA. The fourth site is a Patient Service Center at Ängelholm hospital where patients come for blood and urine sampling. The samples were then further transported to laboratories for analysis. The staff at the Patient Service Center has the same kind of assignments as the staff at the primary care centers.

The intended users (operators) in this evaluation are assistant nurses with a level of high school education, and no laboratory training. This is equivalent with the expected educational level for intended users at CLIA waived laboratories.

Table 5.2. Operator information

Site	Operator	Age	Gender	Education
Delfinen Primary Care	**1**	41	female	Assistant nurse
Delfinen Primary Care	**2**	65	female	Assistant nurse
Delfinen Primary Care	**3**	42	female	Assistant nurse
Munka Primary Care	**4**	55	female	Assistant nurse
Munka Primary Care	**5**	55	female	Assistant nurse
Sjöcrona Primary Care	**6**	40	female	Assistant nurse
Sjöcrona Primary Care	**7**	47	female	Assistant nurse
PTC at Ängelholm hospital*	**8**	27	female	Assistant nurse
PTC at Ängelholm hospital*	**9**	31	female	Assistant nurse
PTC at Ängelholm hospital*	**10**	28	female	Assistant nurse
Närlunda Primary Care	**11**	52	female	Assistant nurse

*PTC=Patient Service Center at Ängelholm hospital

5.4.2 Procedure

The intended users were provided with the HemoCue WBC System Quick Reference Guide (Attachment 2), the draft HemoCue WBC Package Insert (Attachment 3), and the HemoCue WBC System Operating Manual (Attachment 4). The assistant nurses did not receive any training, coaching, prompting, written or verbal instructions beyond the written test procedure and the supplied instructions for use.

Each sample was analyzed according to instructions for use with the HemoCue WBC System by the assistant nurse (intended user) and in duplicate with the Sysmex XS-1000*i* by the professional user from HemoCue. All samples were masked, i.e. the operators did not receive any information about the WBC level for the sample.

The evaluation time was 4th of April to 17th of October 2007, during 31 working days.

After the study was completed all the intended users were given a questionnaire in order to evaluate if the participants understood how to use the device correctly and if it was easy to use and whether it followed the operating manual and package insert. The questionnaire contained questions concerning:

1. the microcuvette
2. the analyzer
3. general aspects of the system and the provided instructions for use
4. the overall impression of the system and its simplicity

5.4.3 Sample Material

In total 415 human blood samples were collected and analyzed. The test material used in the study were capillary and venous blood samples:

- Leftover venous blood samples from Clinical Chemistry Laboratories at Ängelholm, Halmstad and Helsingborg Hospitals.
- Capillary blood samples from patients at Delfinen and Munka Primary Care Centers
- Spiked capillary samples from employees at HemoCue AB

5.4.4 Financial Disclosure

See Attachment 6.

5.4.5 Selection of Comparative Method (CM)

The chosen comparative method (CM) is the Sysmex XS-1000*i*. The Sysmex XS-1000*i* is an automated hematology analyzer for *in vitro* diagnostic use in clinical laboratories.

The Sysmex XS-1000*i* is a comparative method of type B which has traceability to the manual light microscopic WBC method (the Reference Method, RM). The mathematical relationship is defined as 1:1, see Table 5.3 and Figure 5.3 below.

Table 5.3. Relationship between CM and RM

WBC level x10^9/L	Number of samples	Mean values, x10^9/L		Difference between methods		SD, x10^9/L		CV, %	
		Sysmex XS-1000i (CM)	Manual light microscopic WBC method (RM)	x10^9/L	%	Sysmex XS-1000i (CM)	Manual light microscopic WBC method (RM)	Sysmex XS-1000i (CM)	Manual light microscopic WBC method (RM)
0-4.0	19	2.52	2.23	0.29	13.0	0.04	0.25	1.6	11.2
4.1-6.5	33	5.46	5.36	0.10	1.9	0.09	0.43	1.6	8.0
6.6-12.0	23	8.98	8.91	0.07	0.8	0.19	0.38	2.1	4.3
> 12.0	34	22.59	22.48	0.11	0.5	0.26	0.50	1.2	2.2
Total	**109**	**11.03**	**10.90**	**0.13**	**1.2**	**0.18**	**0.42**	**1.6**	**3.9**

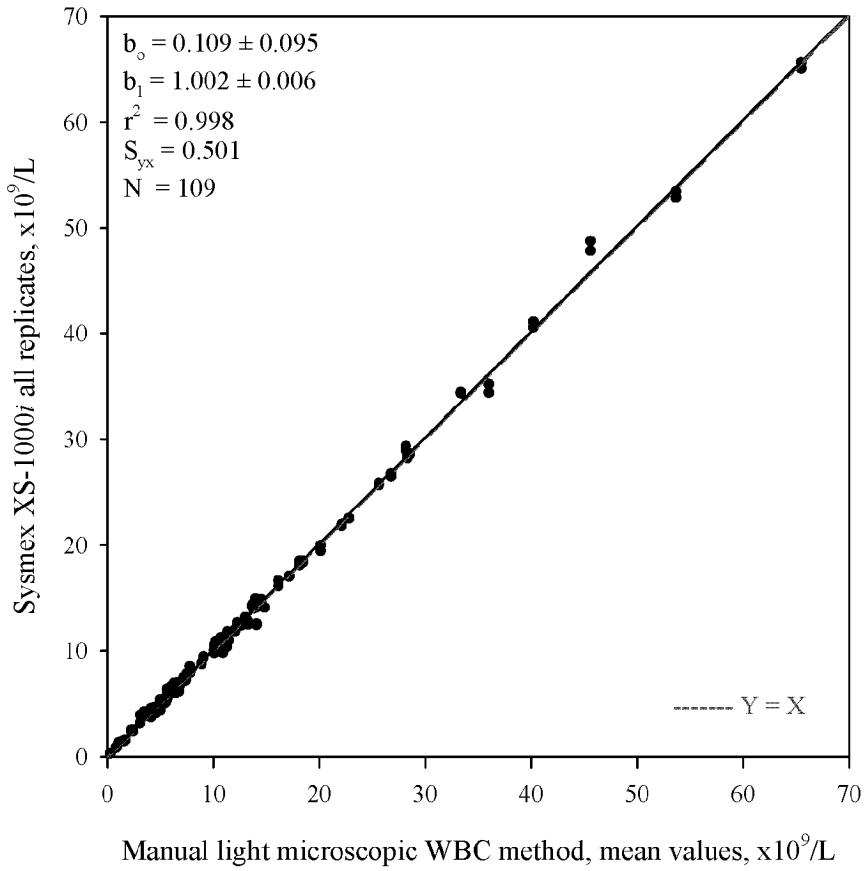


Figure 5.3. Regression analysis, Sysmex XS-1000*i* (CM) and Manual light microscopic WBC method (RM)

5.4.6 *Specimen collection and sample analysis*

Sampling has been done according to CDC Recommendations and Reports "Good Laboratory Practices for Waived Testing Sites - Survey Findings from Testing Sites Holding a Certificate of Waiver Under the Clinical Laboratory Improvement Amendments of 1988 and Recommendations for Promoting Quality Testing" Vol. 54 / RR-13. Note: this is not applicable for the spiked capillary samples from HemoCue.

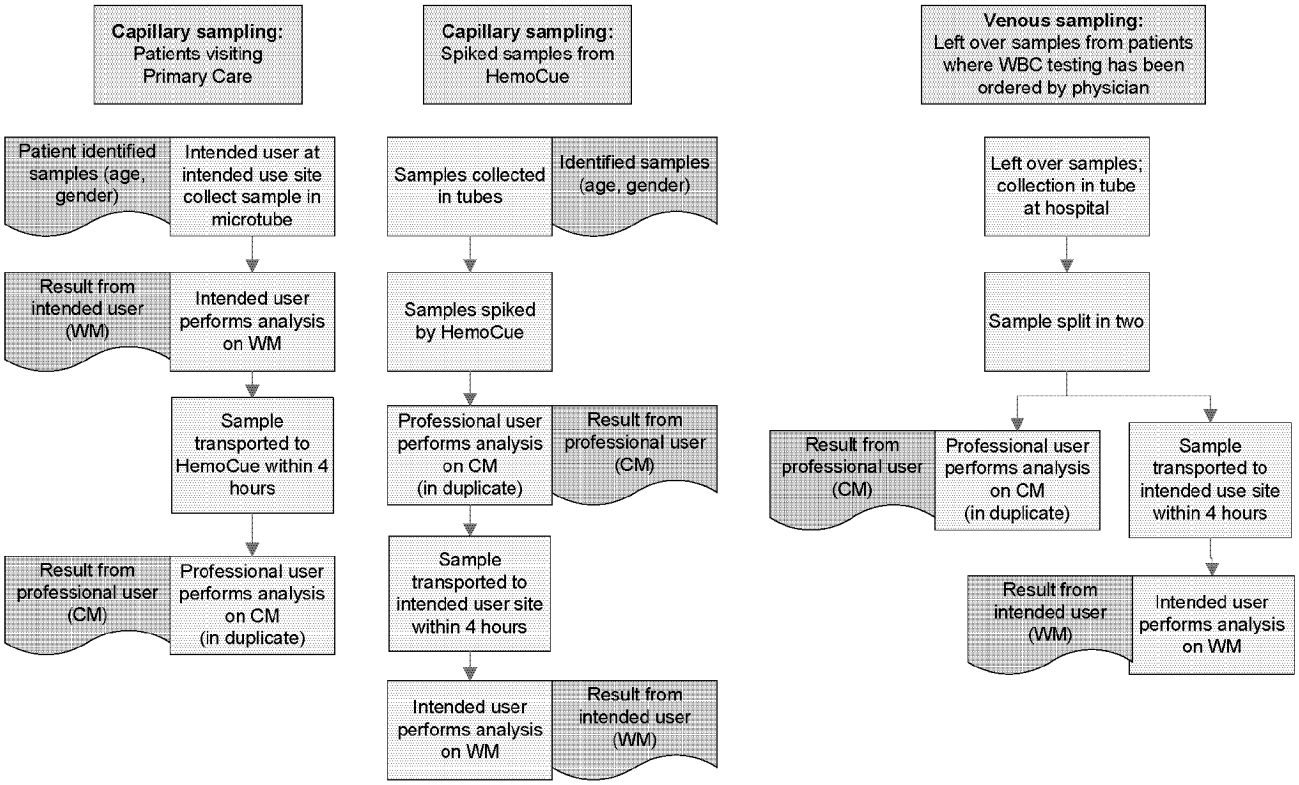


Figure 5.4. Flowchart of the specimen collection and sample analysis used in the field study

5.4.7 *Statistical Analysis (for each site and combined)*

5.4.7.1 ***Statistical Methods***

- Descriptive statistics according to CLIA Draft Guidance of 2005
  - Mean, min, median, max, box-and-whisker plots
  - Scatter plot
- Regression analysis
- Total Analytical Error (TAE)

5.4.7.2 ***Exclusion of Results***

Calculations were performed on 411 samples. For samples P37, S12 and H6 the sample volume were too small for analysis with Sysmex XS-1000*i*. Sample H49 showed HHH on the HemoCue WBC system. Measurements above 30.0 x10^9/L are displayed as "HHH" on the HemoCue WBC. The mean value on Sysmex XS-1000*i* was 29.475 for sample H49.

5.4.7.3 ***Descriptive Statistics***

The samples have been divided into the medical relevant intervals based on the mean values of the comparative method, i.e. Sysmex XS-1000*i*.

Table 5.4. Summary raw data Multicenter study

WBC x10^9/L	Number of samples	% of total	Sysmex XS-1000*i*, x10^9/L				HemoCue WBC, x10^9/L		
			Mean values	Min values	Median values	Max values	Min values	Median values	Max values
≤ 5.0	112	27	2.86	0.34	2.79	5.02	0.4	2.7	13.3
5.1-10.0	181	44	7.29	5.09	7.11	10.04	4.2	7.2	11.5
10.1-30.0	118	29	18.15	10.06	17.19	28.74	9.7	17.3	29.0
Total:	**411**	**100**	**9.20**	**0.34**	**7.19**	**28.74**	**0.4**	**7.4**	**29.0**

Table 5.5. Summary raw data Delfinen Primary Care

WBC x10^9/L	Number of samples	% of total	Sysmex XS-1000*i*, x10^9/L				HemoCue WBC, x10^9/L		
			Mean values	Min values	Median values	Max values	Min values	Median values	Max values
≤ 5.0	37	30	3.15	0.85	2.98	4.96	1.0	2.8	13.3
5.1-10.0	45	36	7.54	5.35	7.38	10.04	4.8	7.6	11.5
10.1-30.0	42	34	18.08	10.21	16.53	27.91	11.0	17.2	28.7
Total:	**124**	**100**	**9.80**	**0.85**	**7.75**	**27.91**	**1.0**	**8.2**	**28.7**

Table 5.6. Summary raw data Munka Primary Care

WBC x10^9/L	Number of samples	% of total	Sysmex XS-1000*i*, x10^9/L				HemoCue WBC, x10^9/L		
			Mean values	Min values	Median values	Max values	Min values	Median values	Max values
≤ 5.0	34	22	2.80	1.18	2.74	5.01	1.3	2.9	4.7
5.1-10.0	79	52	7.24	5.09	7.03	9.96	4.2	7.1	10.6
10.1-30.0	39	26	17.44	10.06	17.08	28.61	9.7	17.3	28.5
Total:	**152**	**100**	**8.86**	**1.18**	**7.12**	**28.61**	**1.3**	**7.3**	**28.5**

Table 5.7. Summary raw data Sjöcrona Primary Care

WBC 10^9/L	Number of samples	% of total	Sysmex XS-1000*i*, x10^9/L				HemoCue WBC, x10^9/L		
			Mean values	Min values	Median values	Max values	Min values	Median values	Max values
≤ 5.0	18	26	2.79	0.72	2.44	4.50	0.7	2.6	5.3
5.1-10.0	31	44	7.13	5.32	7.06	10.04	4.2	7.0	10.0
10.1-30.0	21	30	17.83	10.20	15.69	28.50	10.9	16.8	29.0
Total:	**70**	**100**	**9.22**	**0.72**	**7.15**	**28.50**	**0.7**	**7.4**	**29.0**

Table 5.8. Summary raw data Patient Service Center

WBC 10^9/L	Number of samples	% of total	Sysmex XS-1000*i*, x10^9/L Mean values	Min values	Median values	Max values	HemoCue WBC, x10^9/L Min values	Median values	Max values
≤ 5.0	19	32	2.54	0.34	2.34	5.02	0.4	2.3	4.8
5.1-10.0	26	43	7.22	5.25	6.93	9.89	4.4	6.8	10.2
10.1-30.0	15	25	20.55	12.80	20.02	28.74	13.0	18.2	27.0
Total:	**60**	**100**	**9.07**	**0.34**	**6.74**	**28.74**	**0.4**	**6.4**	**27.0**

Table 5.9. Samples analyzed at Närlunda Primary Care (too few results to calculate statistics for this subgroup, but the data are included in Table 5.4 above.)

Sample	Sysmex XS-1000*i* ,x10^9/L repl 1	repl 2	mean	HemoCue WBC x10^9/L
KH16	18.91	19.33	19.12	18.7
KH17	2.04	2.07	2.06	2.1
KH18	2.25	2.61	2.43	2.7
KH32	2.39	2.48	2.44	2.6
KH33	3.39	3.57	3.48	3.7

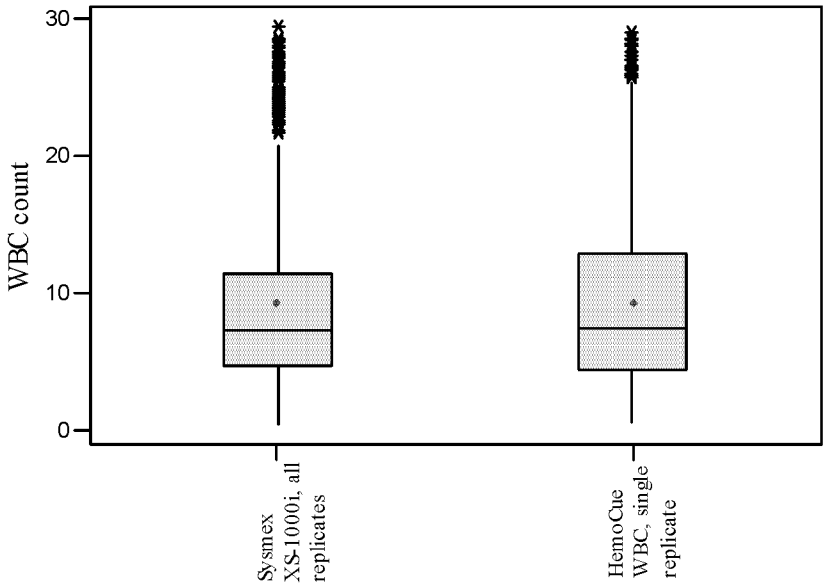


Figure 5.5. Box and whiskers plot for Multicenter study, HemoCue WBC and Sysmex XS-1000*i*, x10^9/L.

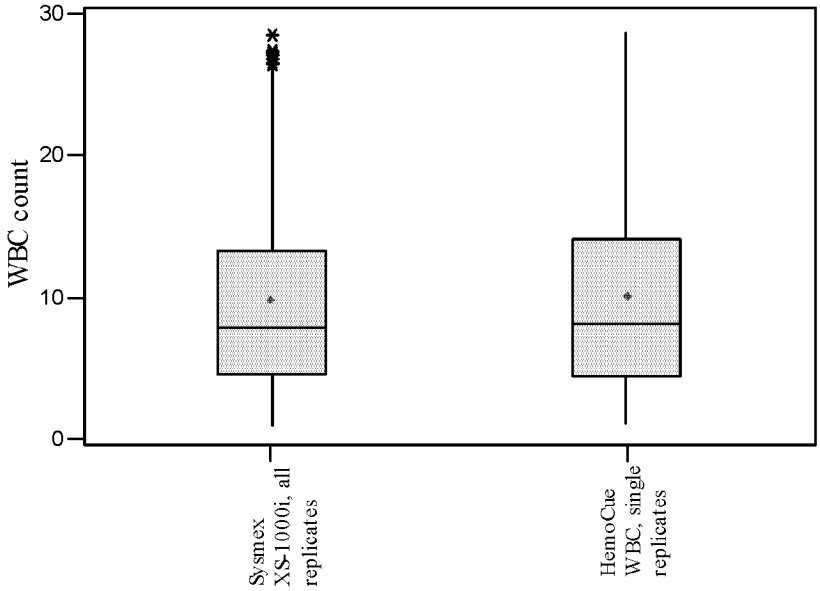


Figure 5.6. Box and whiskers plot for Delfinen Primary Care Center, HemoCue WBC and Sysmex XS-1000*i*, x10^9/L.

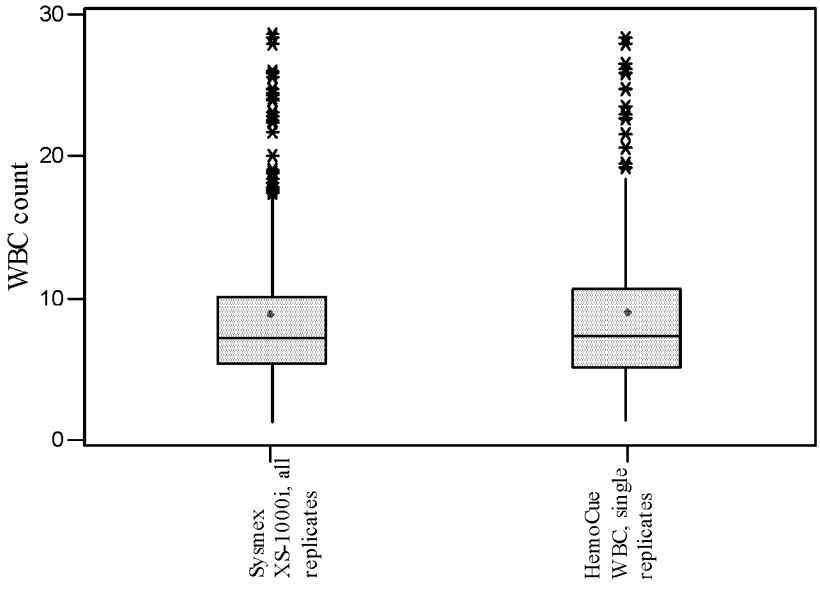


Figure 5.7. Box and whiskers plot for Munka Primary Care Center, HemoCue WBC and Sysmex XS-1000*i*, x10^9/L

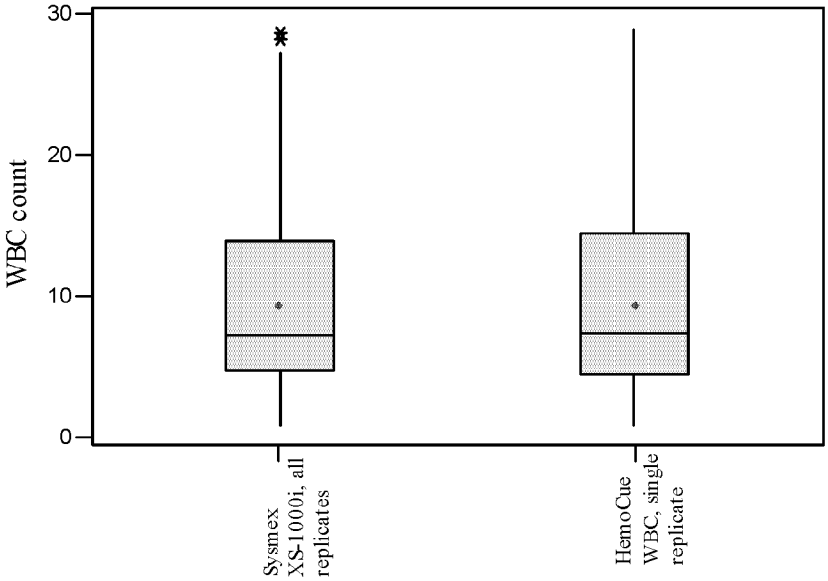


Figure 5.8. Box and whiskers plot for Sjöcrona Primary Care Center, HemoCue WBC and Sysmex XS-1000*i*, x10^9/L.

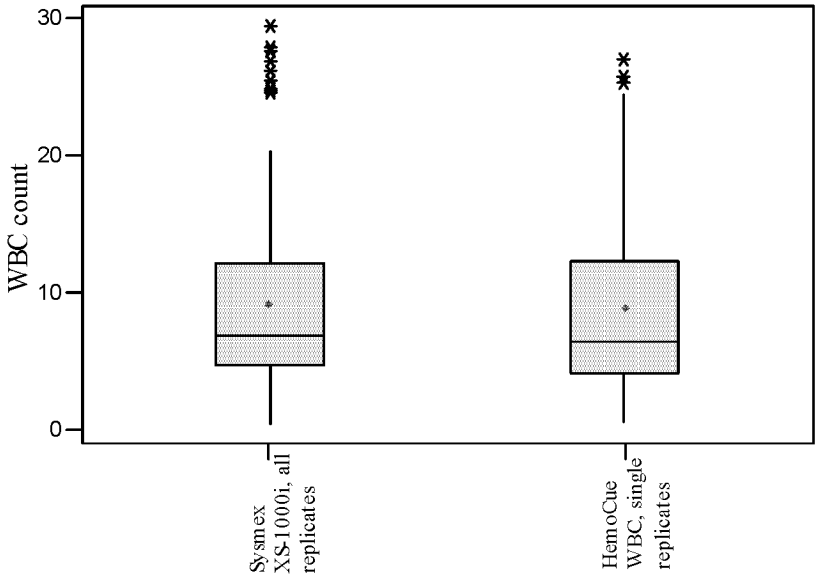


Figure 5.9. Box and whiskers plot for Patient Service Center, HemoCue WBC and Sysmex XS-1000*i*, x10^9/L.

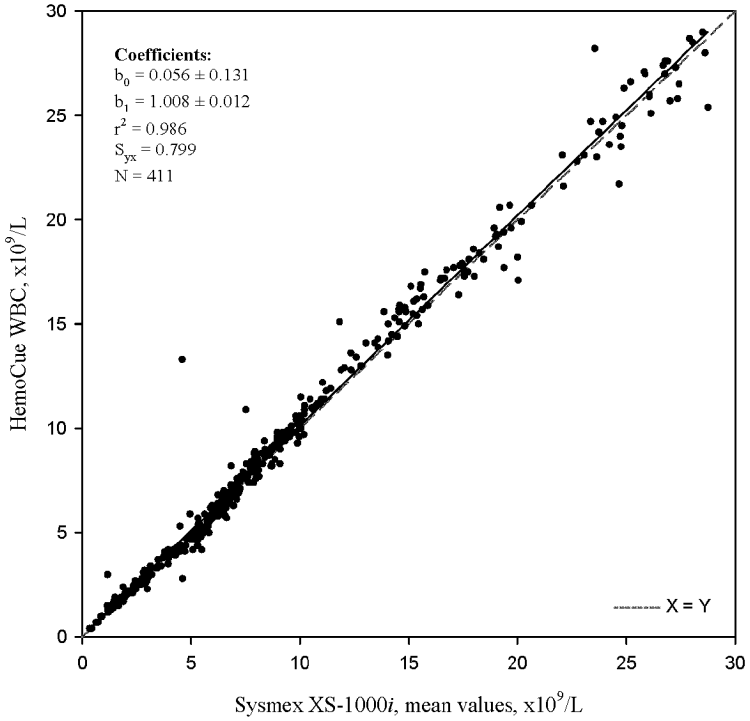


Figure 5.10. Regression analysis and scatter plot for Multicenter study, HemoCue WBC vs Sysmex XS-1000*i*.

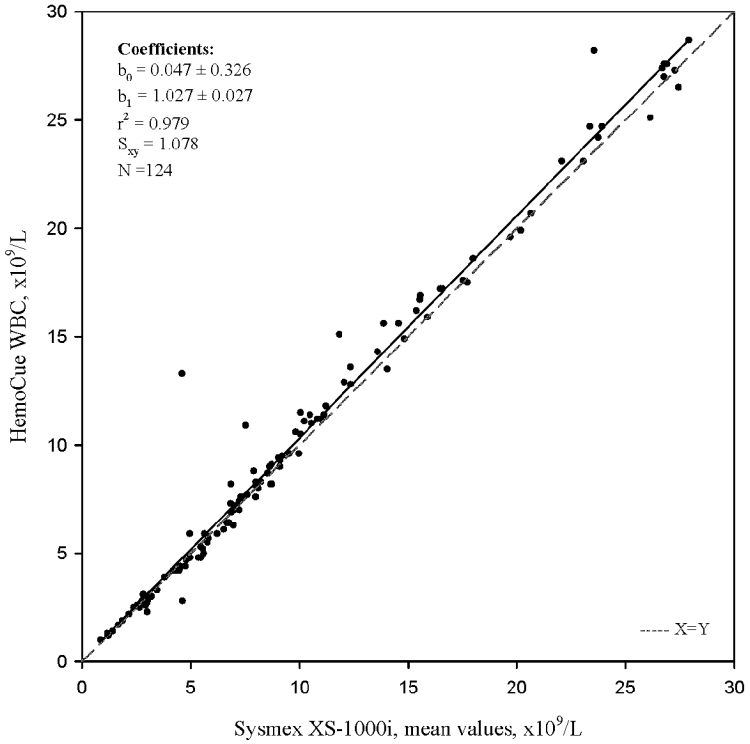


Figure 5.11. Regression analysis and scatter plot for Delfinen Primary Care Center, HemoCue WBC vs Sysmex XS-1000*i*.

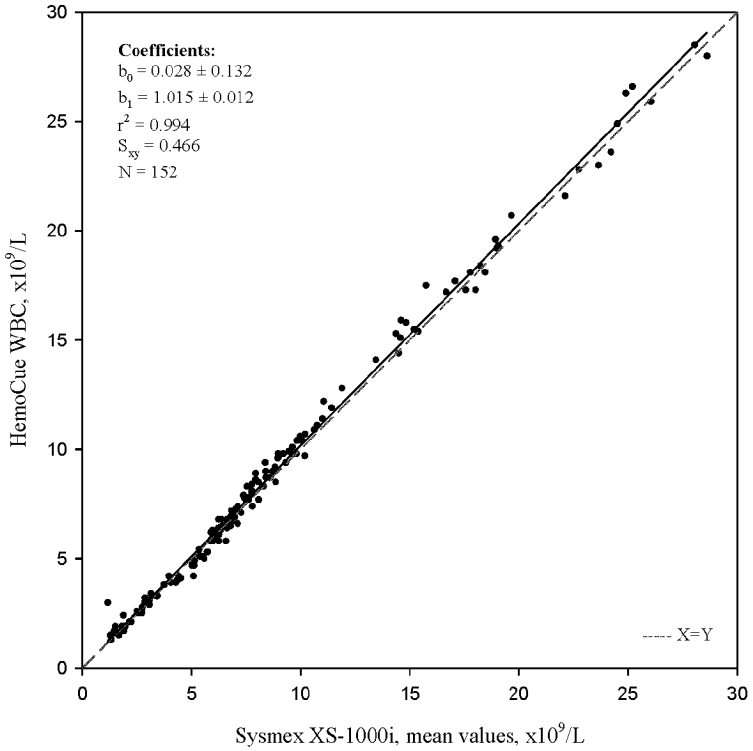


Figure 5.12. Regression analysis and scatter plot for Munka Primary Care Center, HemoCue WBC vs Sysmex XS-1000*i*.

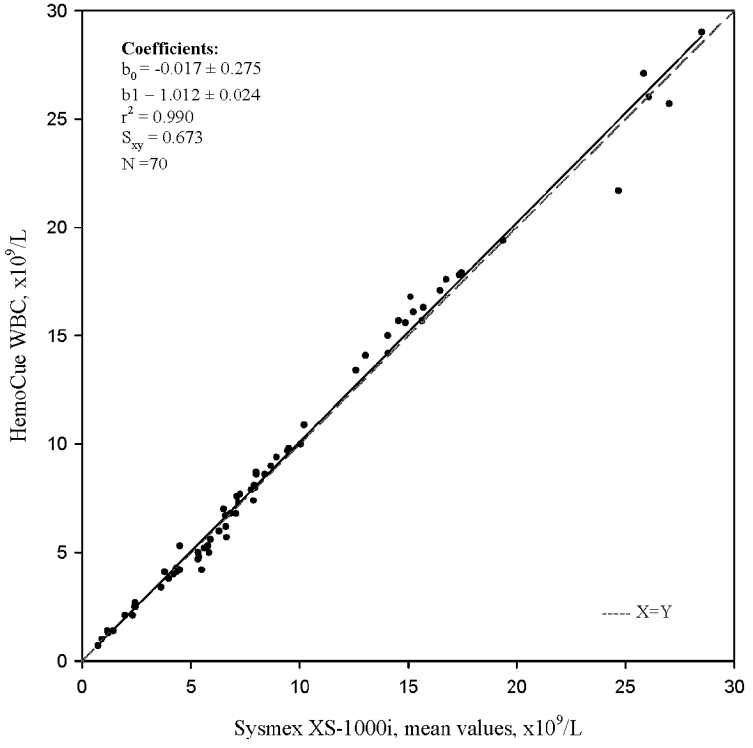


Figure 5.13. Regression analysis and scatter plot for Sjöcrona Primary Care Center, HemoCue WBC vs Sysmex

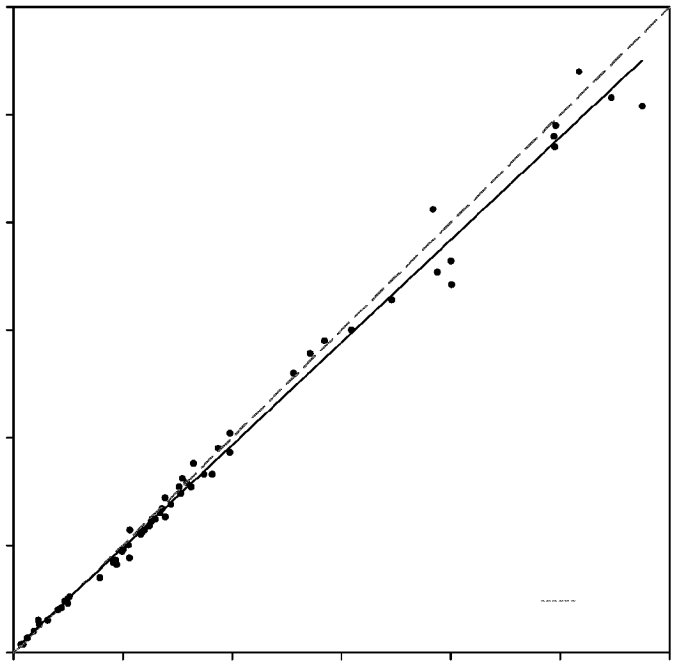


Figure 5.14. Regression analysis and scatter plot for Patient Service Center, HemoCue WBC vs Sysmex XS-1000*i*.

5.4.8 Total Analytical Error

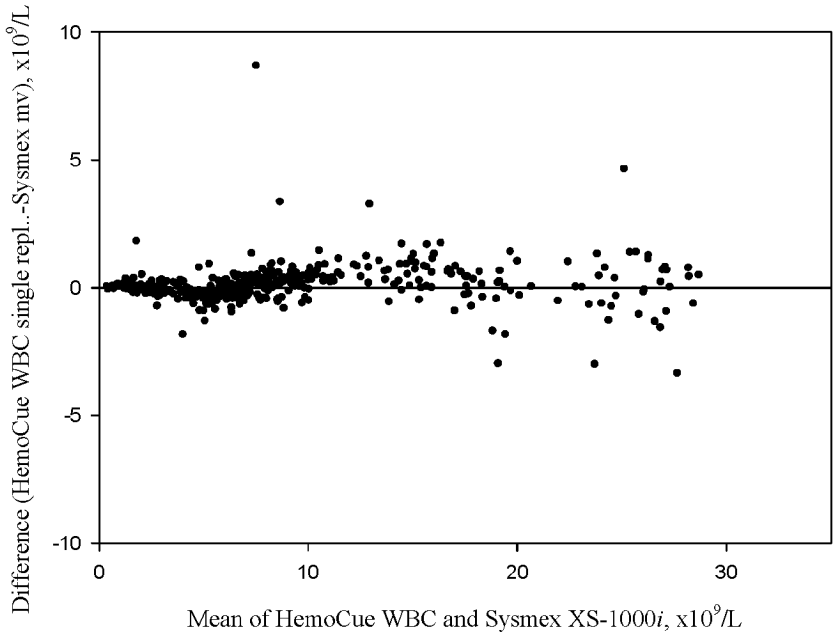


Figure 5.15. Bland-Altman plot for HemoCue WBC user vs Sysmex XS-1000*i*, absolute differences, x10^9/L

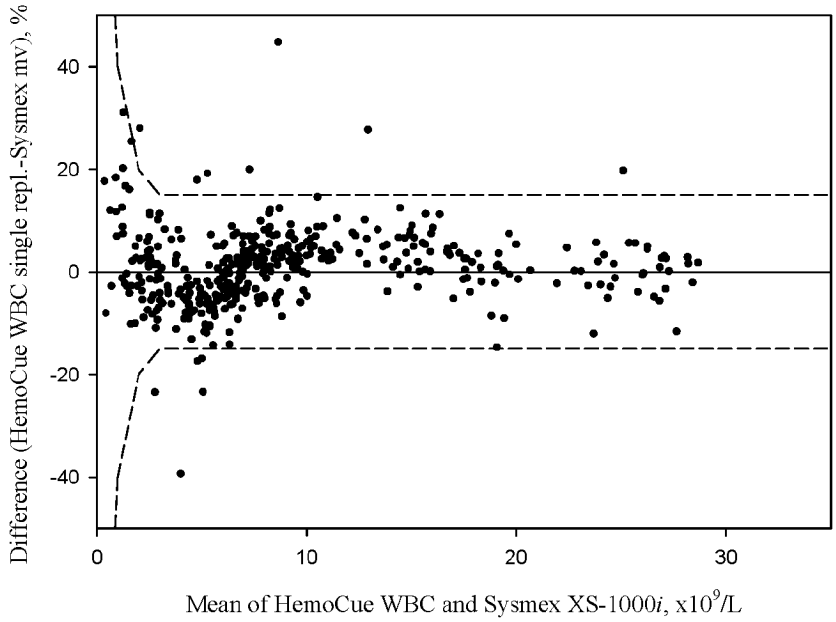


Figure 5.16. Bland-Altman plot for HemoCue WBC user vs Sysmex XS-1000*i*, relative differences, %. Sample KM18 (155.3%) and H20 (189.1%) are excluded in this plot.

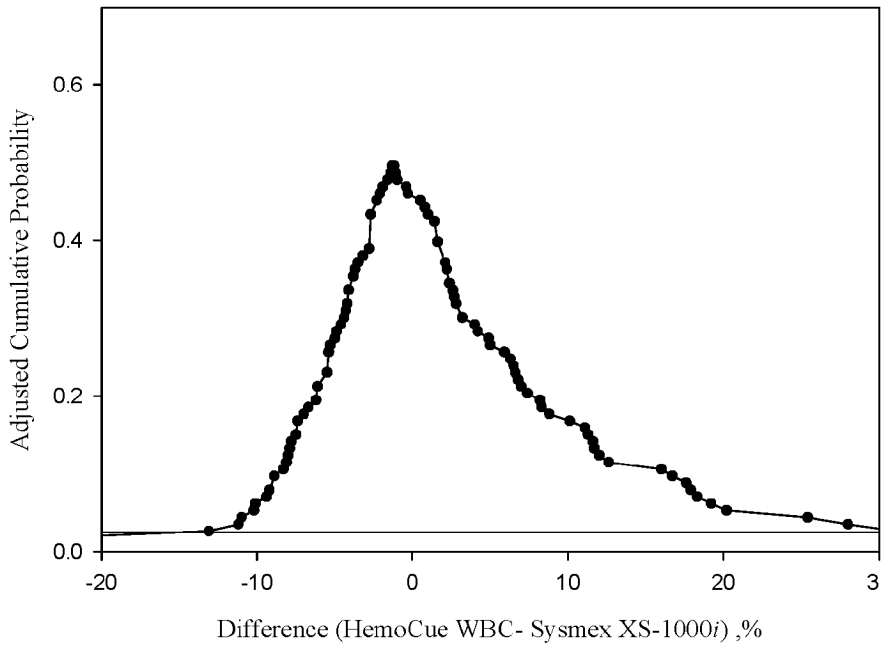


Figure 5.17. Mountain plot for low range. No acceptance limits (±0.4 x10^9/L, ± 15%) has been included in this figure. Sample KM18 (155.3%) and H20 (189.1%) are excluded in this plot.

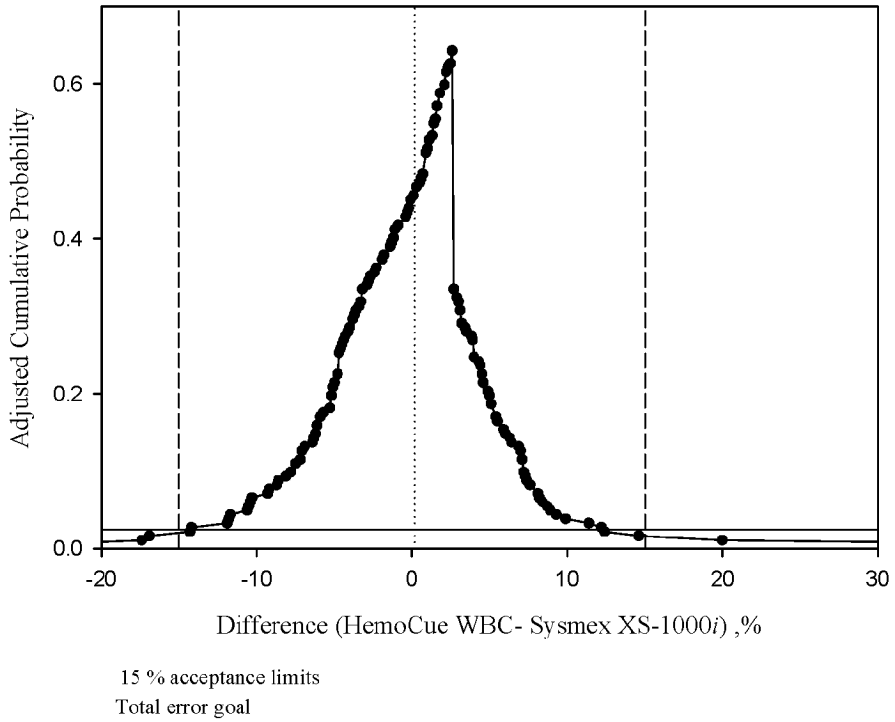


Figure 5.18. Mountain plot for medium range.

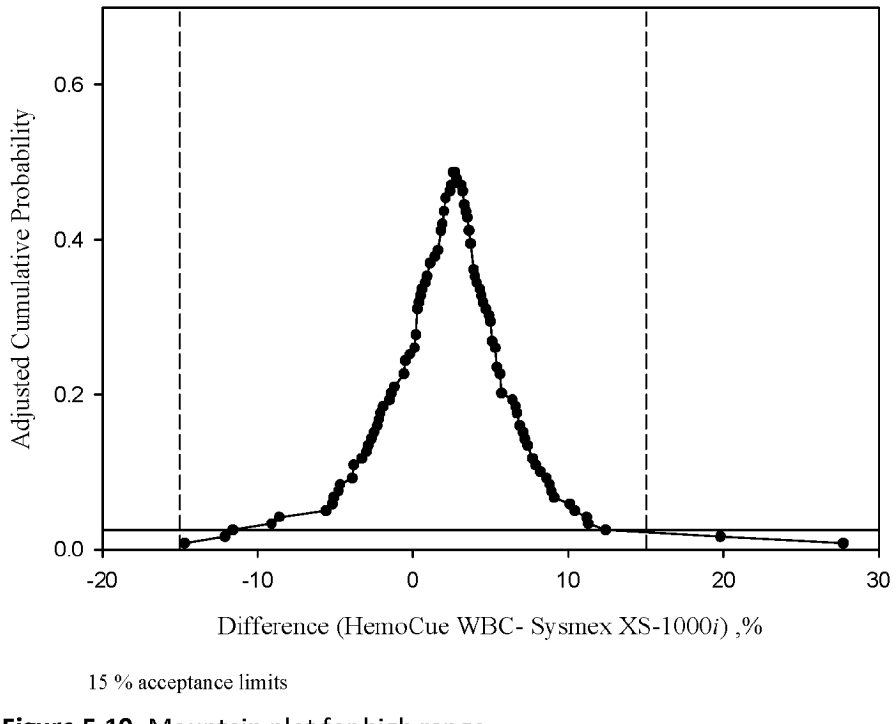


Figure 5.19. Mountain plot for high range.

5.4.9 Allowable Total Error (ATE)

The analytic quality goal for WBC is established in several publications including CLIA to allow a total error (ATE) at 15% [Ref. 1, 2, 3, 4, 5].

Suggestion of clinical action limits has been made in CLSI (NCCLS) H26-A[Ref. 6]:
Clinical Relevance: Abnormal Results May Reflect the Following Conditions

< 3 x10^9/L	Sepsis, chemotherapy, radiotherapy, agranulocytosis, marrow hypoplasia, cobalamin, folate, iron deficiency.
>12 x10^9/L	Acute stress (including surgery), infection, malignancy, lymphoma, leukemia.

5.4.9.1 Establishment of an Error Grid for WBC

There exists no consensus error grid for WBC comparable to the Clarke error grid for glucose. According to the new CLIA guidelines, HemoCue therefore has established a grid based on patient risk and clinical relevance.

A total leukocyte count is measured in different situations in healthcare:

- One of the most common situations is to determine if an infection is bacterial or viral, if the patient should be treated with antibiotics or not. Usually a cut-off value of 12 x10^9 g/L is used. It could be a severe condition for the patient if a false low result is received and the "true" WBC is very elevated.
- Different types of acute leukemia would be critical to miss. Patients with acute leukemia have a massive increase of leucocytes. Getting a normal or only a slightly elevated number of leucocytes missing acute leukemia would be fatal.
- Another possible situation is to monitor the WBC during chemotherapy or other cytotoxic treatment. A value of 3 x10^9 g/L serves as a decision level to permit continuation of the treatment. Small differences here could cause relative severe conditions. Especially missing a patient with a WBC below 1 x10^9 g/L or even worse <0.5 x10^9 g/L because of the increased infection risk.

With this background, HemoCue established the following error grid with zones of ATE and LER:

- ATE: < ±15% or ±0.4 x10^9 g/L the allowable-total-error of deviation. 95% of all samples should fall within this zone.
- LER: 0% of the samples may fall into these zones. The upper left LER zone corresponds to a risk to miss patients with leucopenia (<3 x10^9 g/L) during chemotherapy or other cytotoxic treatment. The lower LER zone corresponds to a risk to miss severe infections or leukemia.

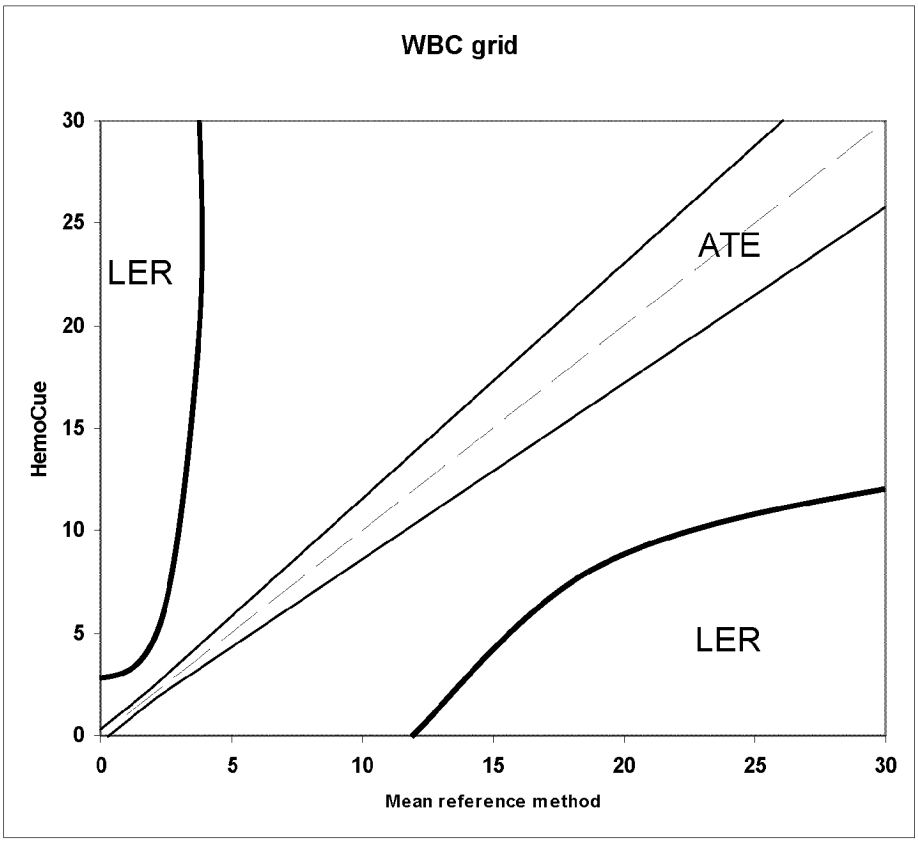


Figure 5.20. Established WBC Error Grid

Literature references to support establishment of WBC error grid:

1. Fraser C.G., Hyltoft Petersen P., Quality goals in external quality assessment are best based on biology, Scand J Clin Lab Invest 1993; 53 suppl 212. Chapter I. Quality planning
2. Fraser C.G., The Necessity of Achieving Good Laboratory Performance, Diabetic Medicine 1990; 7: 490–493.
3. Fraser G.F. and Petersen P.H., Analytical Performance Characteristics Should Be Judged against Objective Quality Specifications., Clin Chem 1999; 45:3, 321–323.
4. Ricós C. et al., Current databases on biological variation, Scand J Clin Lab Invest 1999, 59: 491–500.
5. Sebastián-Gambaro M.Á. et al., Intra- and Inter- Individual Biological Variability Data Bank, Eur J Clin Chem Clin Biochem 1997; 35 (11): 845–852
   This data bank is also available on the following Internet address: http://www.westgard.com
6. NCCLS H26-A Performance Goals for the Internal Quality Control of Multichannel Hematology Analysers; Approved Standard. Vol. 9 No. 9. December 1996.

5.4.9.2 *Result Field Study - ATE*

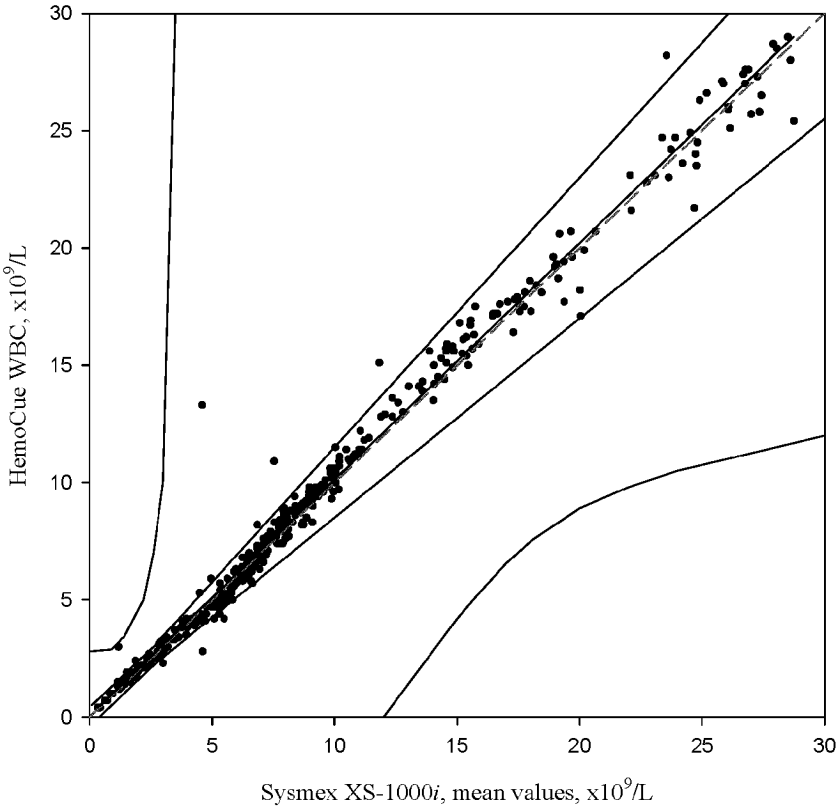


Figure 5.21. Scatter plot in Error grid, HemoCue WBC vs Sysmex XS-1000*i*, x10^9/L

Table 5.10. Number of samples within ATE and LER for each WBC interval and totally

WBC x10^9/L	Number of samples	% of samples within ATE	% of samples within LER
≤ 5.0	112	93.8	0.0
5.1-10.0	181	97.2	0.0
10.0-30.0	118	98.3	0.0
Total	**411**	**96.6**	**0.0**

5.4.10 Result from Questionnaire

Statement	1 (Strongly disagree)	2	3	4	5 (Totally agree)
***1**. It is easy to fill the cuvette*				2	9
***2**. It is easy to place the cuvette into the analyzer*					11
***3**. It is easy to read the value from the display*					11
***4**. It is easy to understand the instruction for use for the system*				1	10
***5**. It is easy to follow the instruction for use for the system*				1	10
***6**. It is possible to handle the system using only Quick Reference Guide*			1	1	9
***7**. It is easy to find information about how to rectify an error code if the analyzer displays one*				3	8

Statement	1 (Totally agree)	2	3	4	5 (Strongly disagree)
***8**. I needed help the first time I performed the test*					11

5.5 Conclusion of the Field Study

The result from the field study clearly shows that the HemoCue WBC System is simple to use in the hands of the intended user and that there is an insignificant risk of receiving erroneous results. This is based on the facts that 96.6% of all results falls within the ATE-zone and the intended users believes that the device is an easy system to use.

6 Proposed Labelling

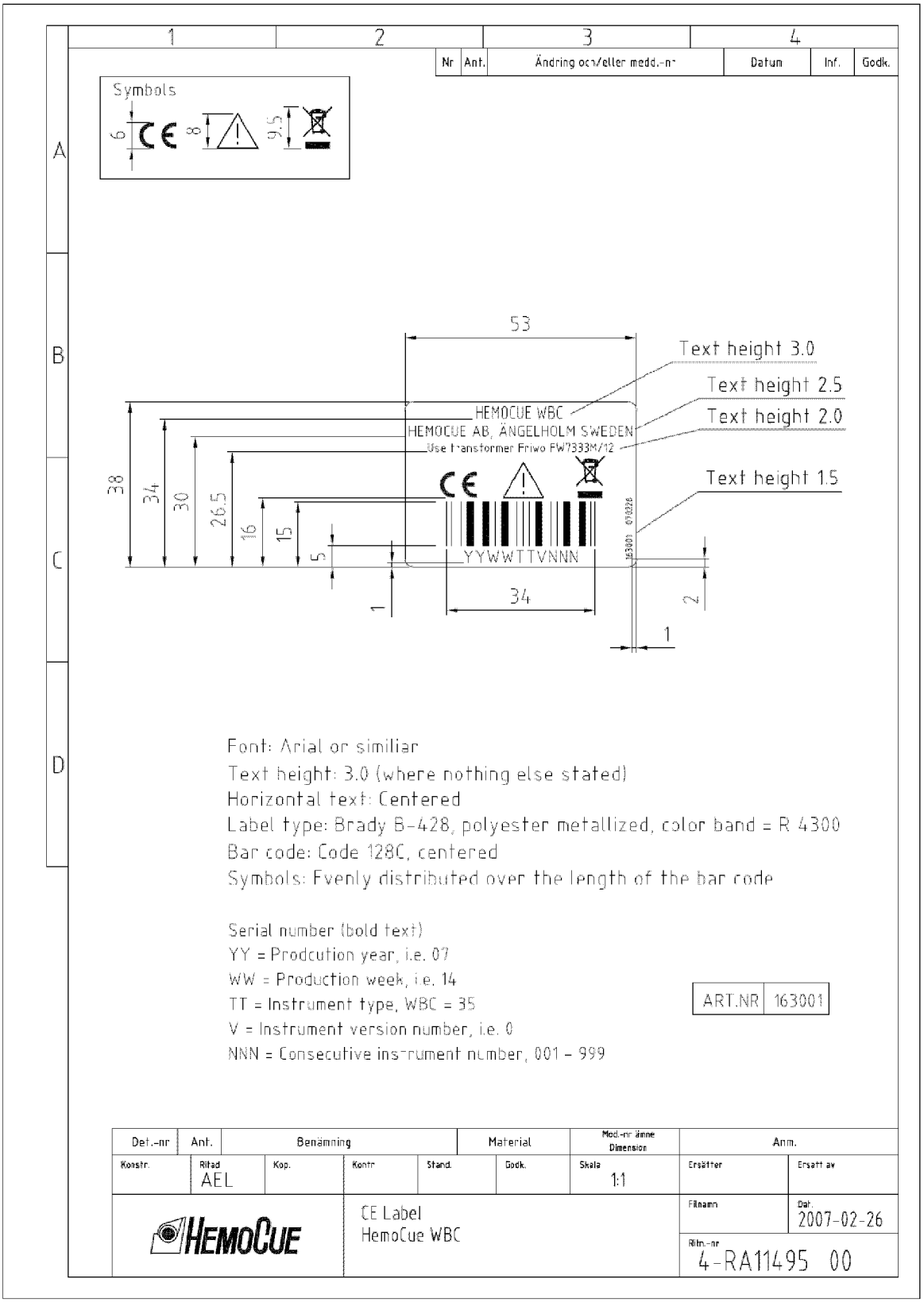
The HemoCue WBC System is delivered to the user including an operating manual, a quick reference guide, a package insert as well as labels shown below. All applicable labelling is presented in this chapter.

Provided with the HemoCue WBC System is:

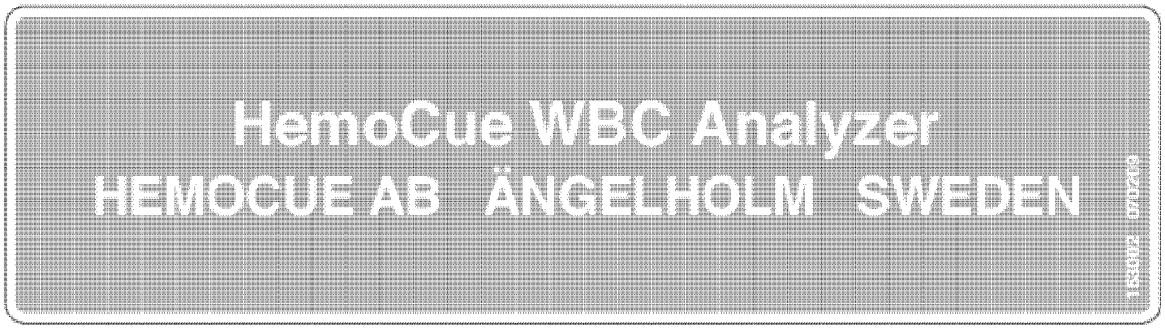
- Quick Reference Guide HemoCue WBC system – provided in Attachment 2
- Package Insert HemoCue WBC Microcuvettes – provided in Attachment 3
- Operating Manual HemoCue WBC Analyzer– provided in Attachment 4
- Training CD HemoCue WBC System - provided in Attachment 5

The HemoCue WBC System utilizes the labels shown on the following pages.

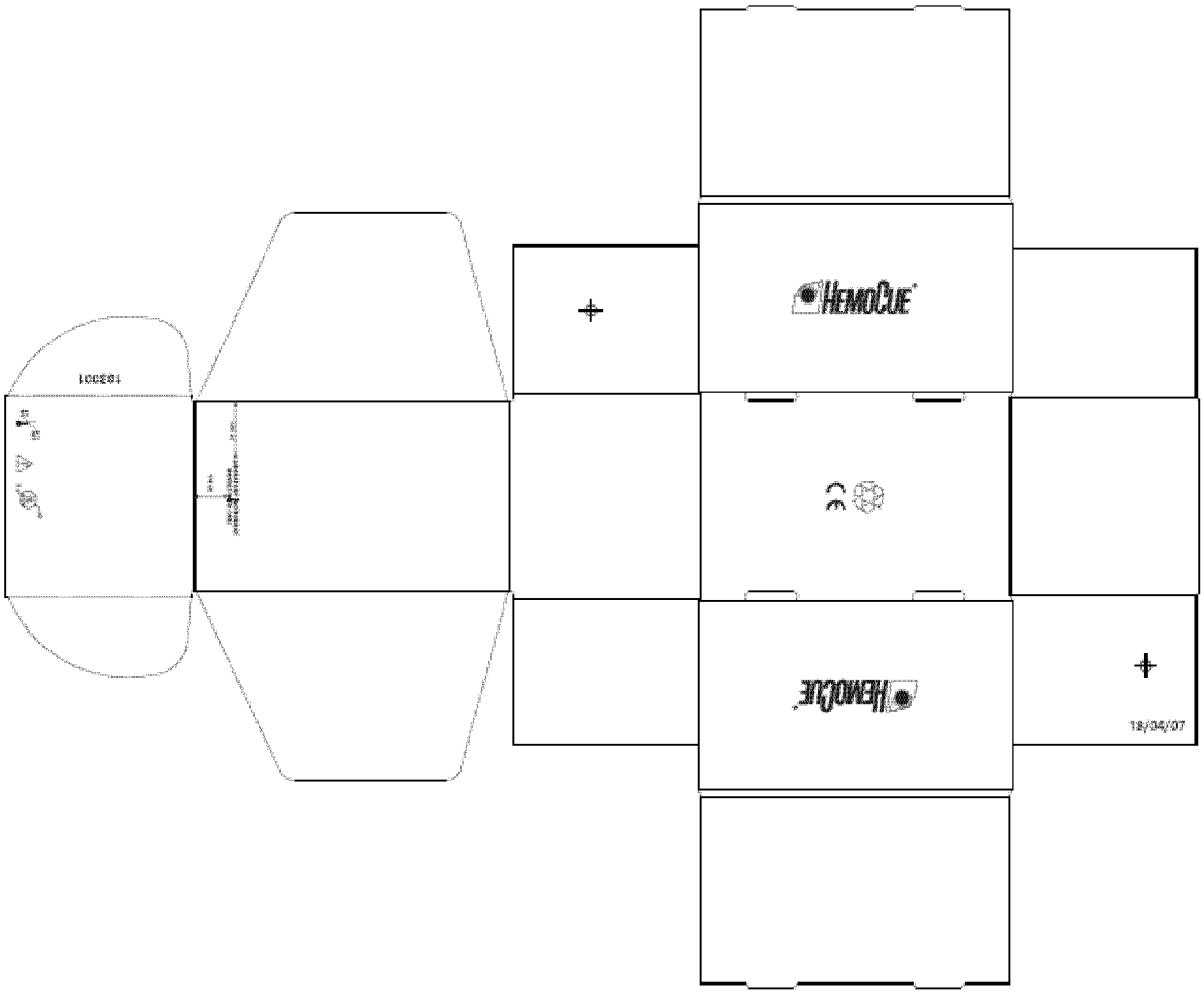
Label, HemoCue WBC Analyzer: Drawing CE and S/N label, box



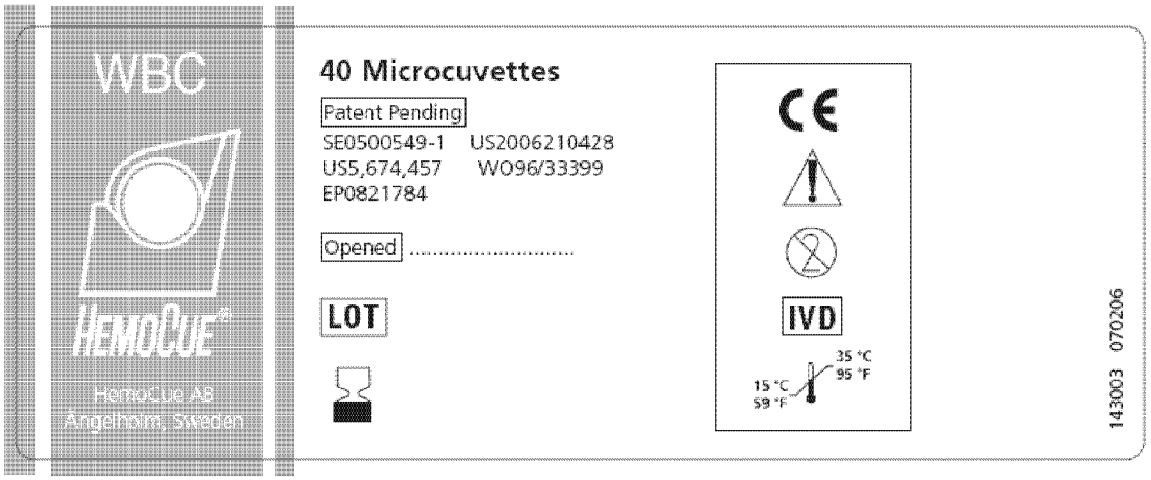
Label, HemoCue WBC Analyzer – box label



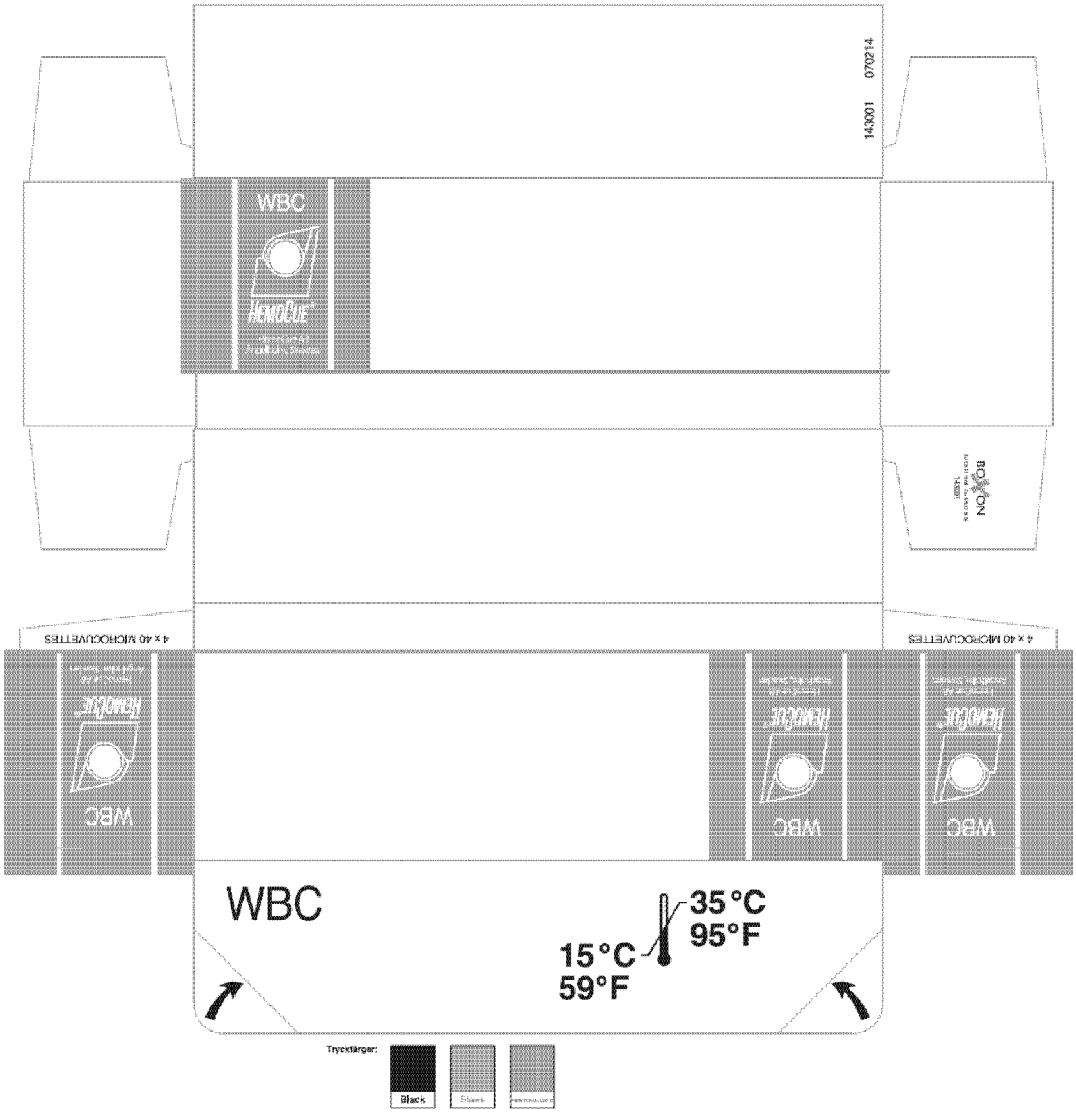
Label, HemoCue WBC Analyzer – box drawing



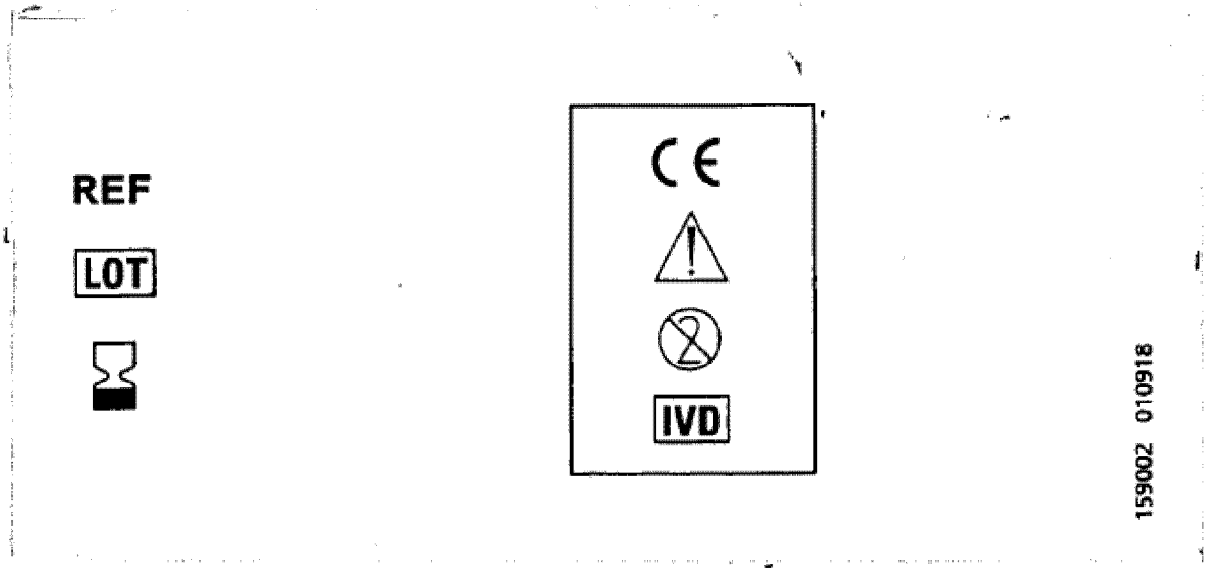
Label, HemoCue WBC Microcuvette – vial label



Label, HemoCue WBC Microcuvette – box drawing



Label, HemoCue WBC Microcuvette – box label (box contains 4 vials)



7 Attachments

Attachment 1 Safety Risk Analysis User and System for HemoCue WBC System -follow up
Attachment 2 Quick Reference Guide HemoCue WBC system
Attachment 3 Package Insert HemoCue WBC Microcuvettes
Attachment 4 Operating Manual HemoCue WBC Analyzer
Attachment 5 Training CD HemoCue WBC System
Attachment 6 Financial Disclosure Statement
Attachment 7 Raw data sheet from field study