Ortho Clinical Diagnostics

Immunonologie en analyses médicales

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13 Février 2018

ORTHO CLINICAL DIAGNOSTIC EUROPEAN CENTRE

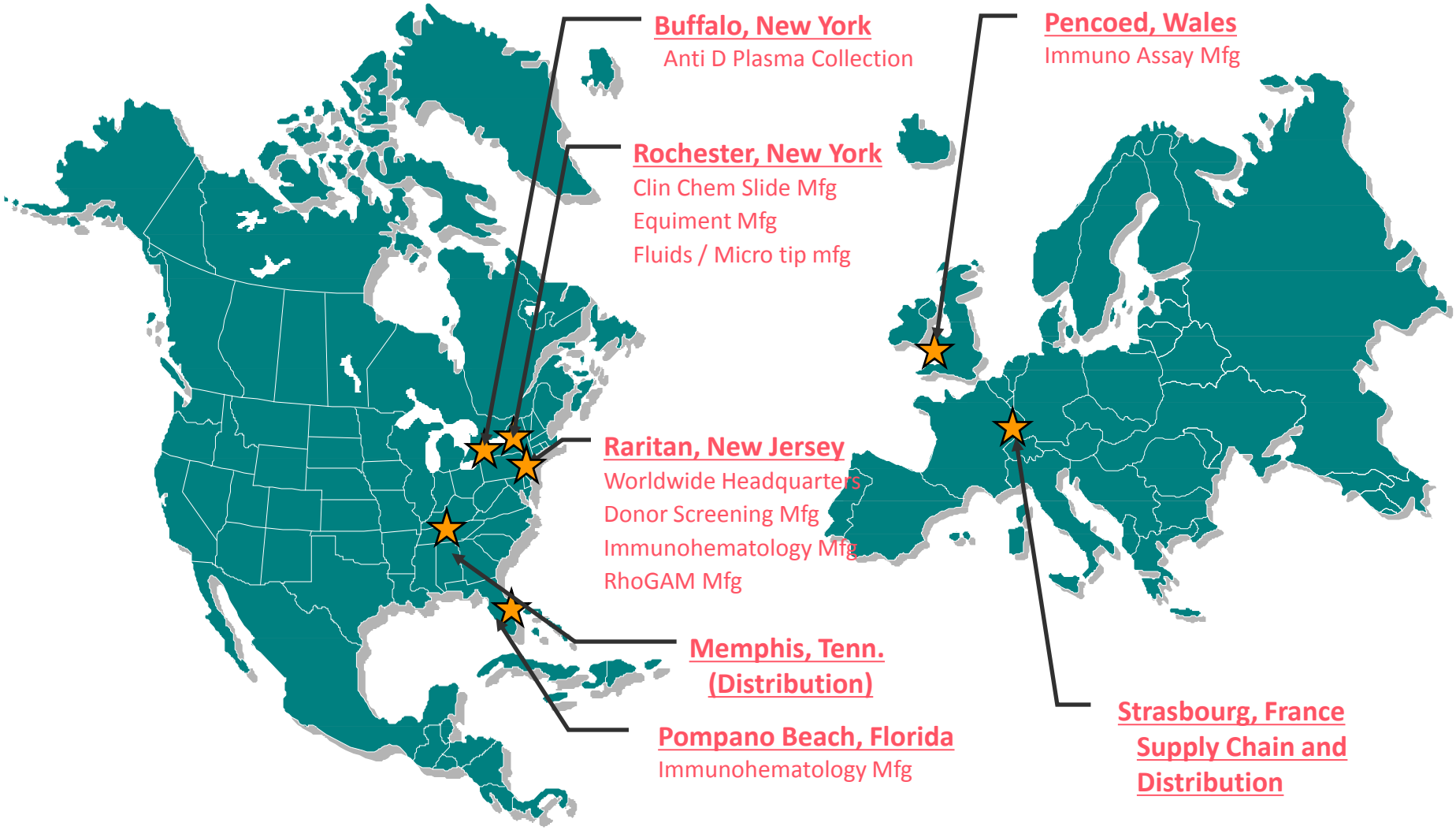
Agenda

Rappel /approfondissement des techniques d'immunomarquage (sandwich versus compétition)

- Principes et techniques immunoenzymologiques :
- Utilisation du couple streptatividine/biotine et amplification du signal
- Immunoturbidimétrie (partie microtips)

  Maladies auto-immunes : exemple avec recherche du facteur rhumatoïde
- Nouveauté : immunocapture-Elisa (dosages IgM en sérologie)
- Exemples d'application dans les domaines de l'immunoanalyse et de la biochimie (Exemples de panels: Virologie, Thyroïde, Anémie, Cardiaque…)
- Notion de validation : interne au fabricant avec panels de séroconversion et échantillons positifs dilués.
- Contrôle des techniques de sérologie : contrôle quotidien

OCD Key Facilities



Buffalo, New York
Anti D Plasma Collection

Rochester, New York
Clin Chem Slide Mfg
Equiment Mfg
Fluids / Micro tip mfg

Raritan, New Jersey
Worldwide Headquarters
Donor Screening Mfg
Immunohematology Mfg
RhoGAM Mfg

Memphis, Tenn. (Distribution)

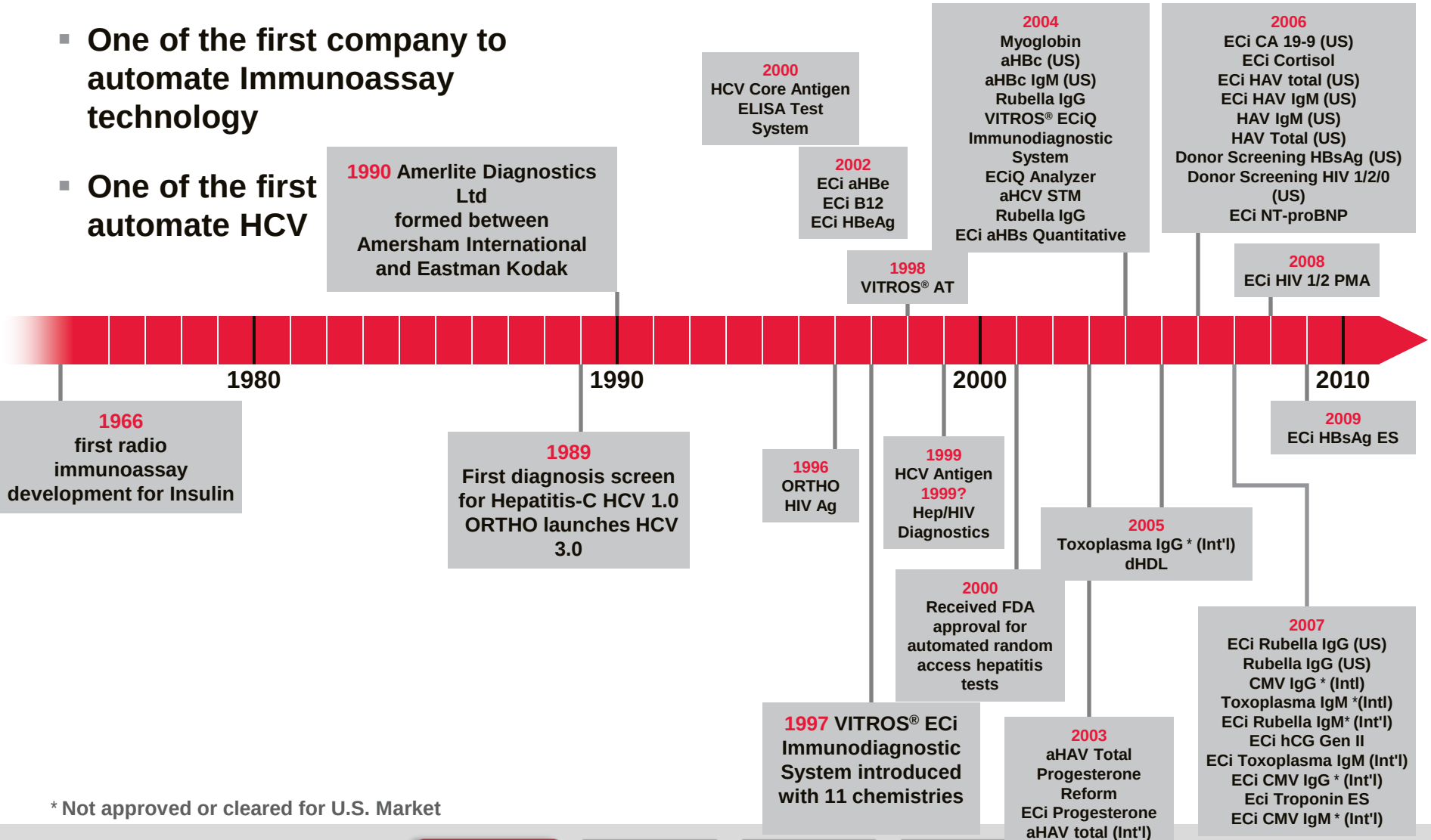
Pompano Beach, Florida
Immunohematology Mfg

Pencoed, Wales
Immuno Assay Mfg

Strasbourg, France
Supply Chain and Distribution

Leadership In Immunoassay

- **One of the first company to automate Immunoassay technology**
- **One of the first automate HCV**



* Not approved or cleared for U.S. Market

VITROS® Immunoassay Performance

SYSTEM DESIGN

- Incubation Times
- Flexible Protocols
- Temperature Control
- Metering Accuracy
- Reliability
- Wash Performance

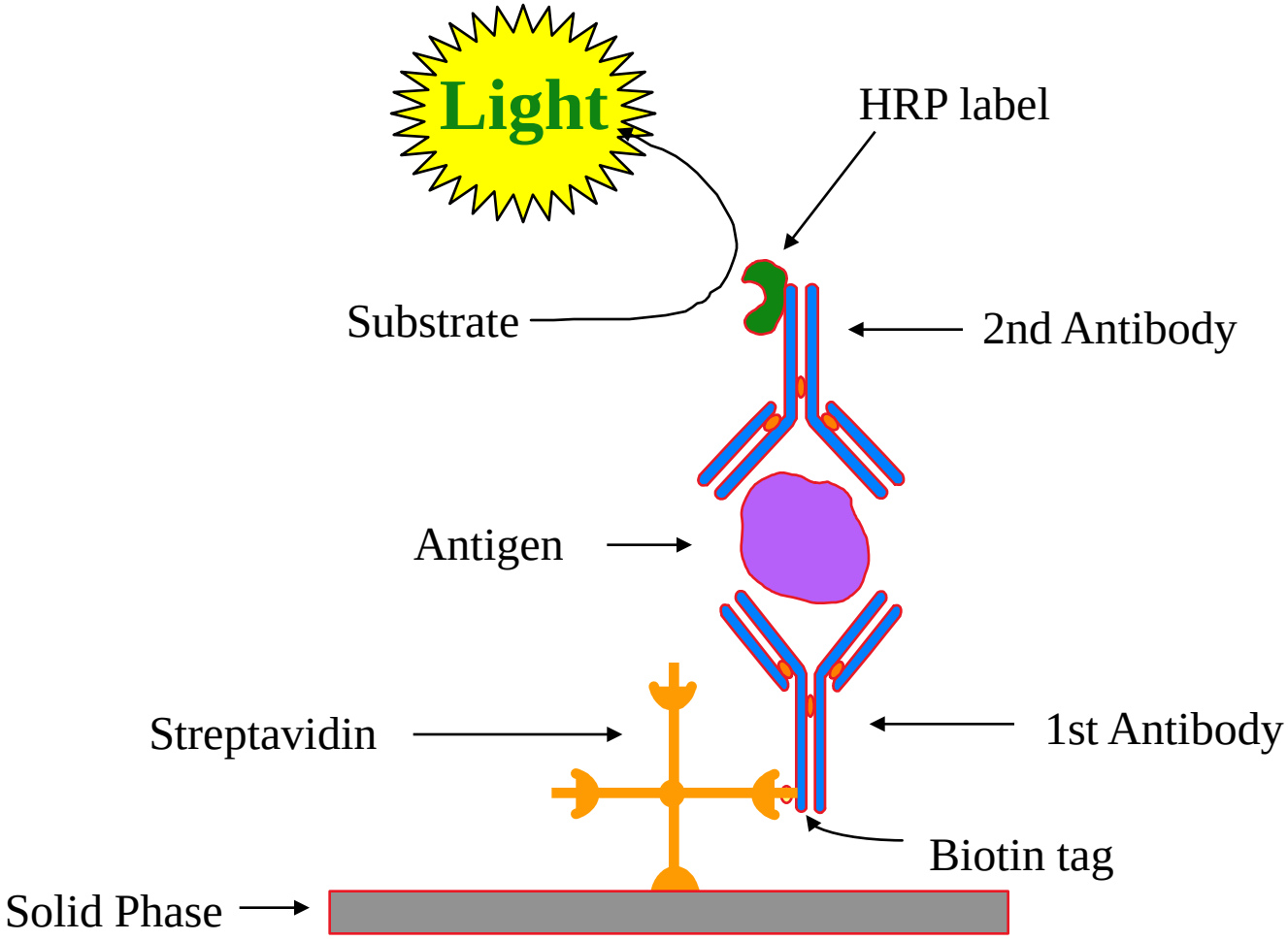
MEASURING TECHNOLOGY

- Enhanced Chemiluminescence
- Micro-Wells
- Range
- Sensitivity
- Precision

ASSAY DESIGN

- Foundation of > 40 years assay design experience
- Antibody Selection Quality
- Purity of Antigen
- Well established & proven technology
- Excellent Raw Material control

Vitros Immunometric Assay on Streptavidin Coated Well



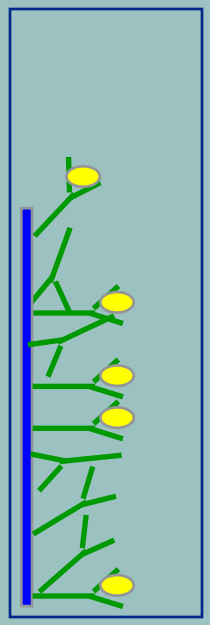
MicroWell

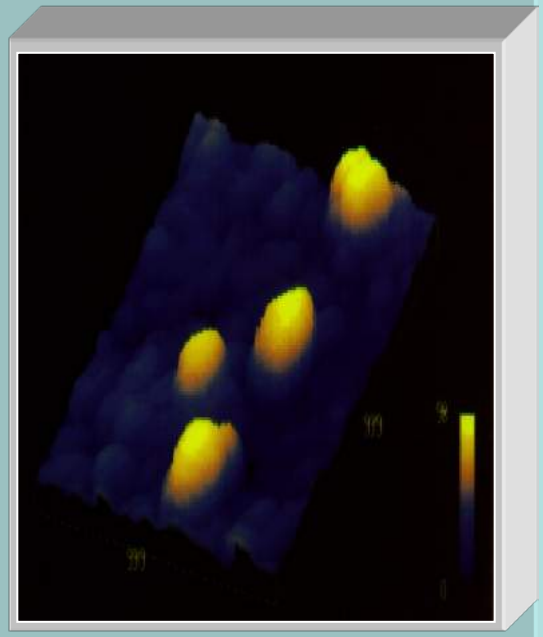
- Provides for excellent assay sensitivity and precision
  - **Consistent and increased binding capacities over conventional passive binding technologies**
  - Coating process increases assay kinetics, binding time and patient sample binding
- Allows for **small sample volumes**
- Minimizes waste because of MicroWell size

La chimiluminescence VITROS ®

Marquage direct du puits

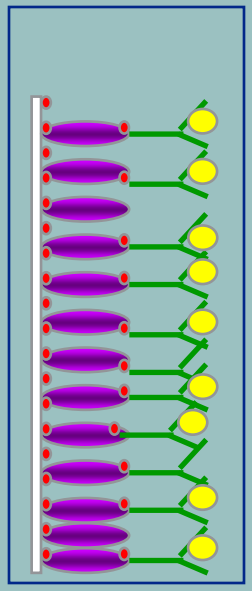
Ac anti Ferritine

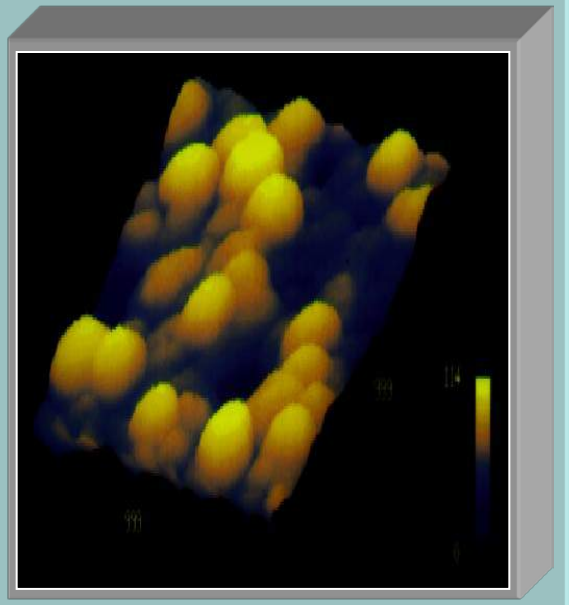




Couple Streptavidine-Biotine:

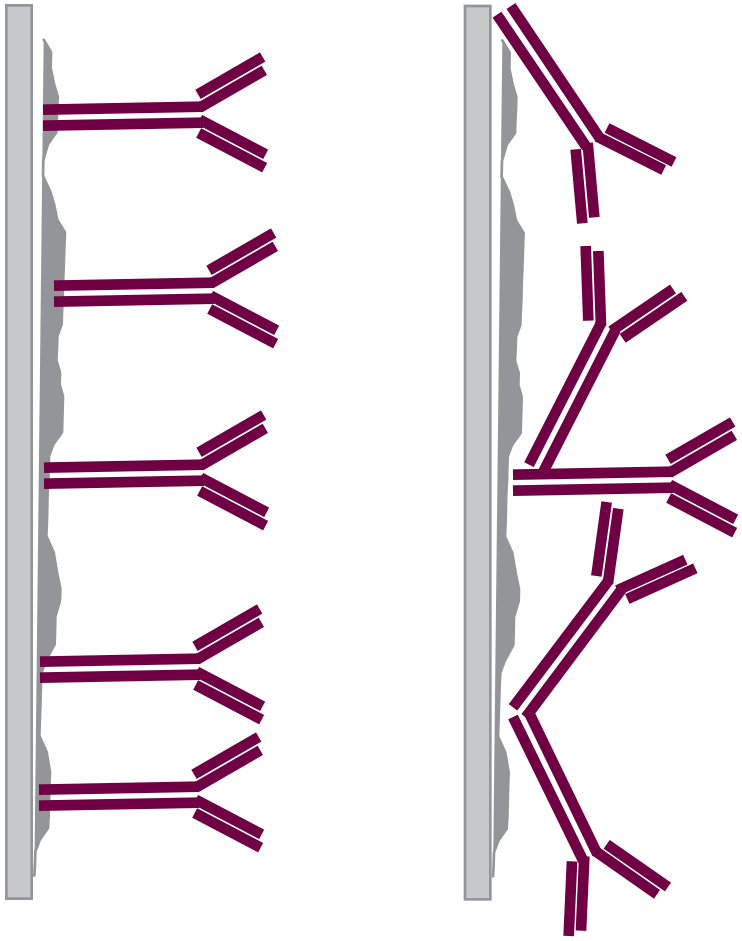
Streptavidine - Ac Anti Ferritine





- **Augmentation x10 du nombre de sites de fixation**
- **cinétique accélérée,**
- **meilleure sensibilité, précision…..**

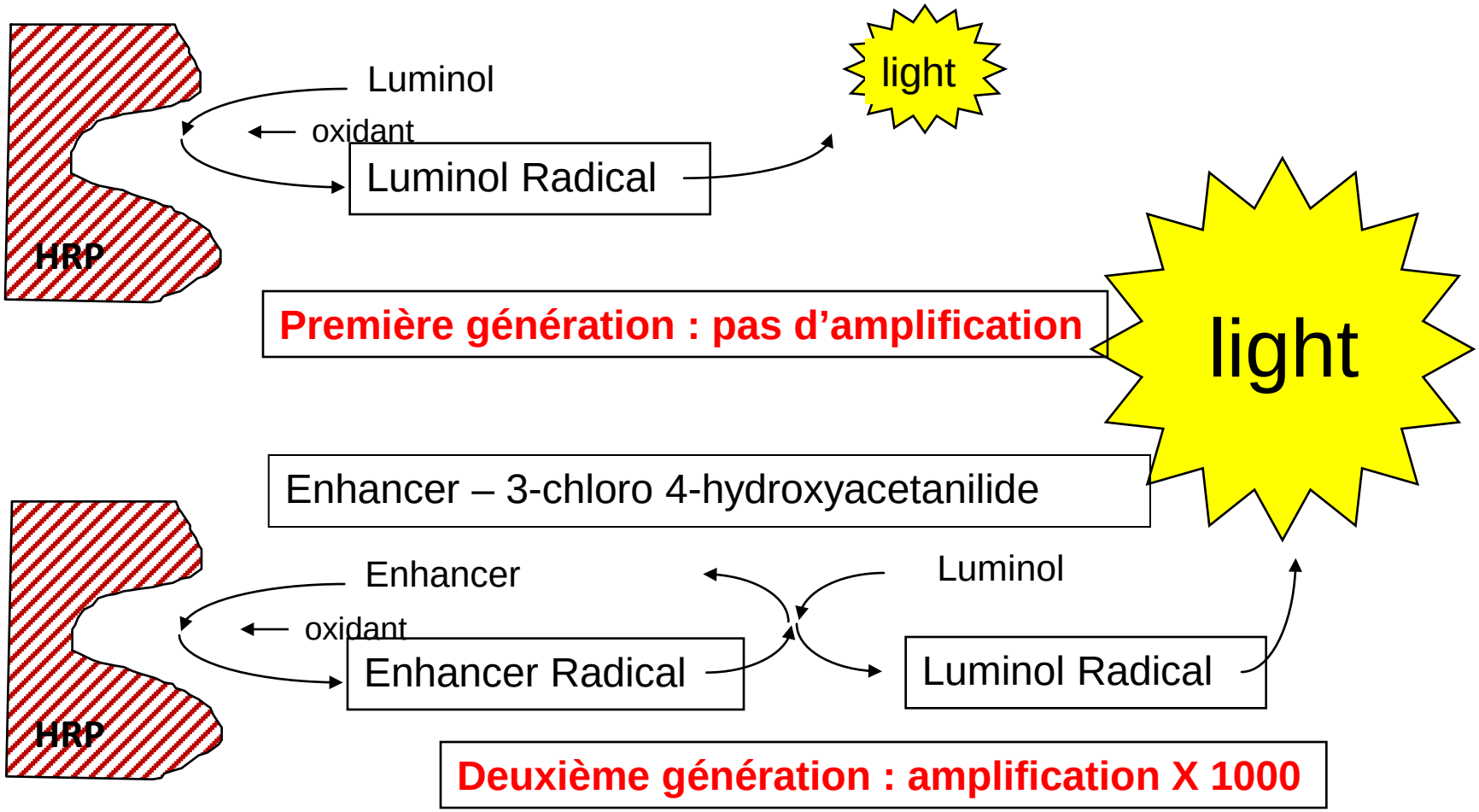
Direct antibody attachment versus SAC technology



TSH assay design

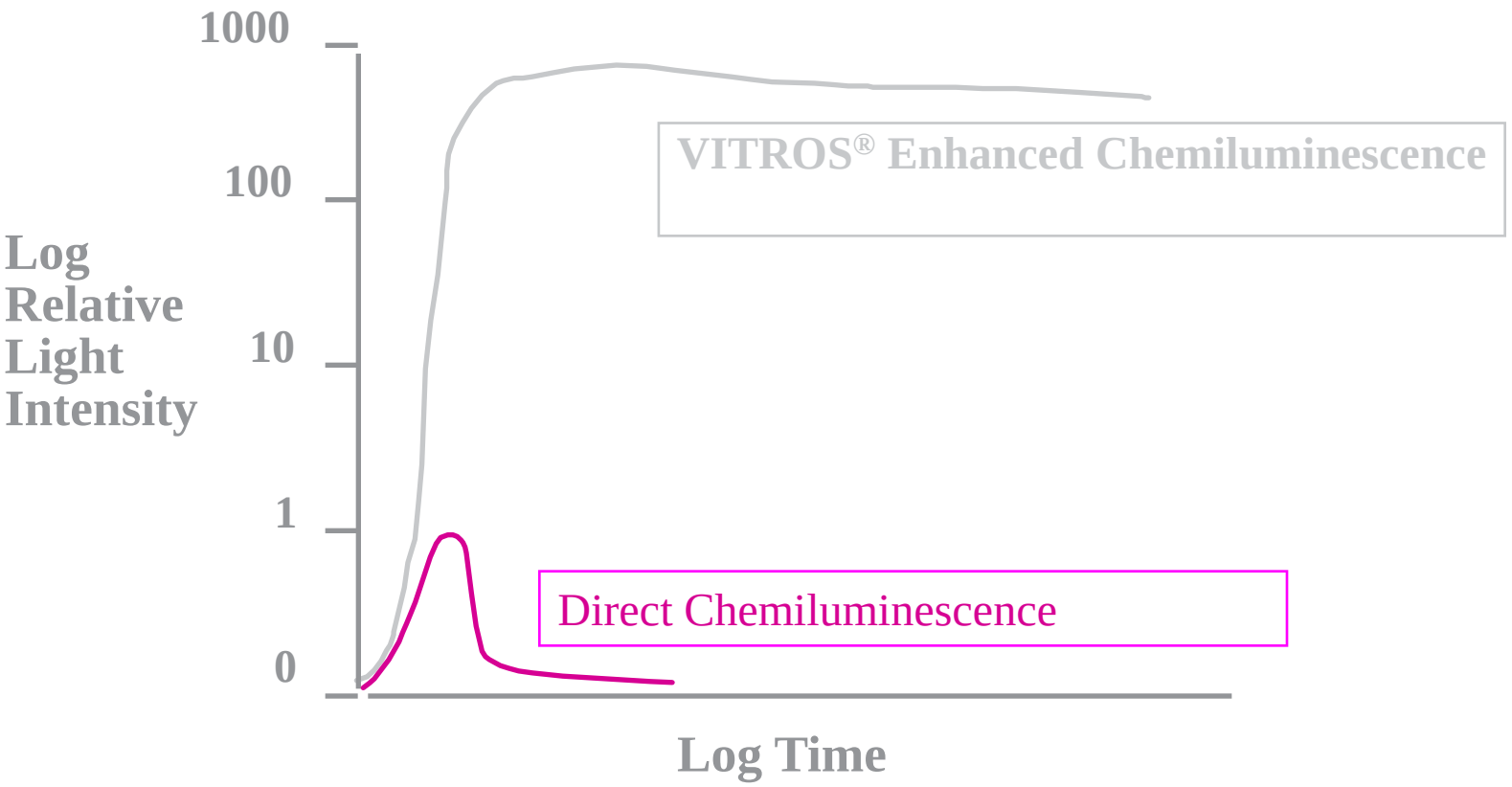
QC	SAC %cv	Direct %cv
low	2.2	7.4
low	2.0	8.2
med	1.7	6.8
med	1.9	7.2
high	1.8	6.4
high	1.8	6.8

La chimiluminescence VITROS ®



Amplification du signal X 1000 pour une meilleure sensibilité

Enhanced Chemiluminescence

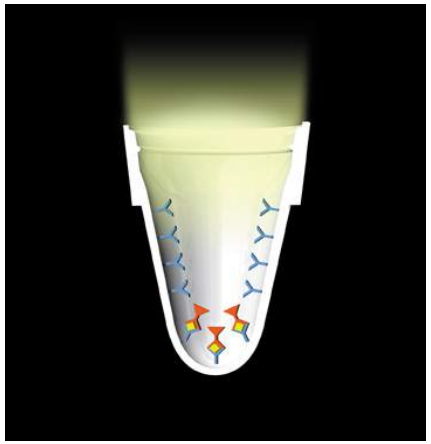


Summers M et al. Luminogenic Reagent Using 3-Chloro 4-Hydroxy Acetanilide to Enhance Perioxidase/LuminolChemiluminescence.*Clinical Chemistry*; 41.S73;1995

Phenols as Enhancers of the Chemiluminescent Horseradish Peroxidase-Luminol-Hydrogen Peroxide Reaction: Application in Luminescence-Monitored Enzyme Immunoassays; Thorpe, Gary H.G.; Kricka, Larry J.; Moseley, Susan B.; Whitehead, Thomas P.;*Clinical Chemistry;* 31:8, 1985

Enhanced Chemiluminescence Detection Technology

Enhanced Chemiluminescence



Integrated dual read mode performs *multiple reads automatically adjusting* to the low or high light output

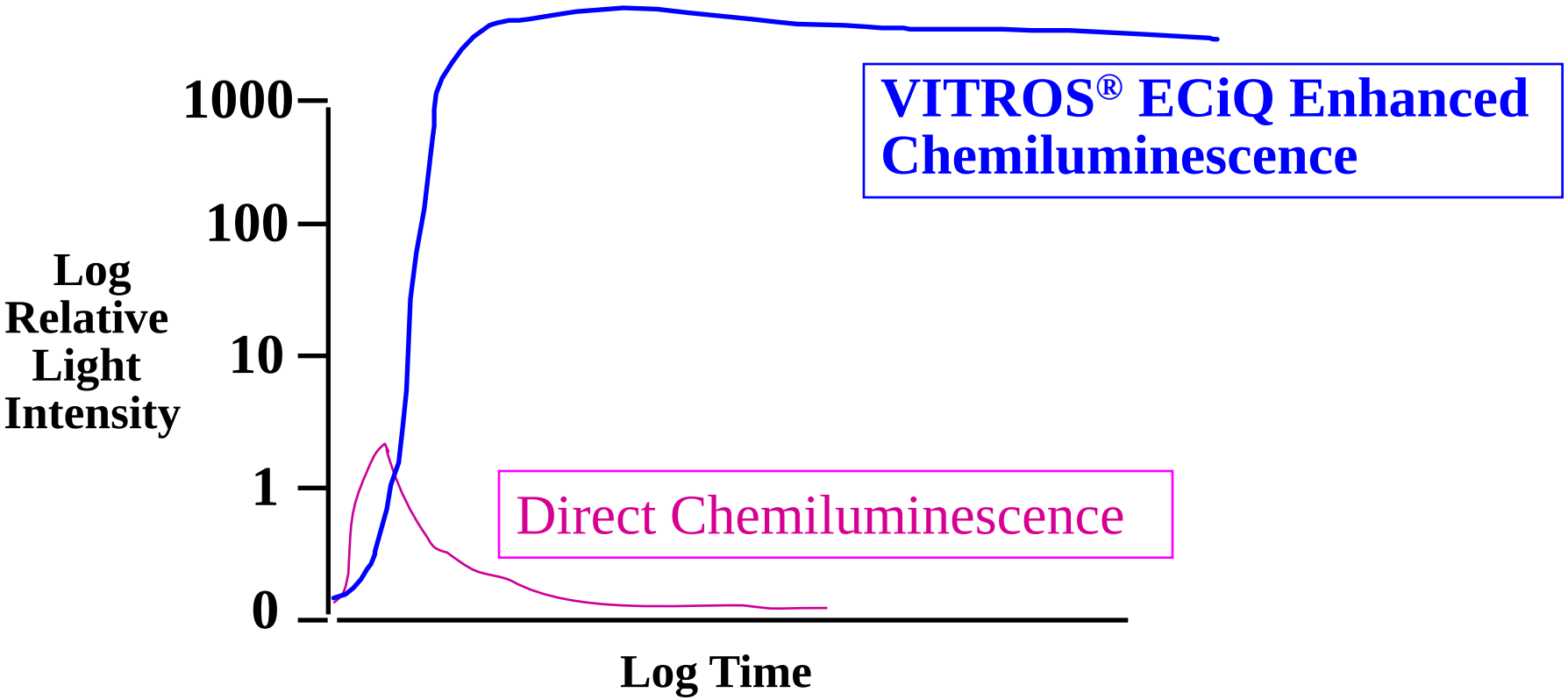
- Compared to direct and other indirect chemiluminescence methods
  - **Improved Signal (Light) Output**
    - Use of patented enhancer produces ***light output at extremely low-analyte concentrations***
    - **Better detection of low analyte concentrations levels**
    - Better medical decisions
  - **Excellent Sensitivity & Precision**
    - More accurate results
    - Ability to capture clinically significant low analyte concentration levels
  - **Broad Dynamic Range**
    - **Less Dilutions & Repeats** - Faster TAT
    - Decreased Costs

Luminogenic Reagent Using 3-Chloro 4-Hydroxy Acetanilide to Enhance Perioxidase/LuminolChemiluminescence.*Clinical Chemistry*; 41.S73;1995

Phenols as Enhancers of the Chemiluminescent Horseradish Peroxidase-Luminol-Hydrogen Peroxide Reaction: Application in Luminescence-Monitored Enzyme Immunoassays; Thorpe, Gary H.G.; Kricka, Larry J.; Moseley, Susan B.; Whitehead, Thomas

integrity by intelli check

Comparison of Indirect with Enhanced Chemiluminescence



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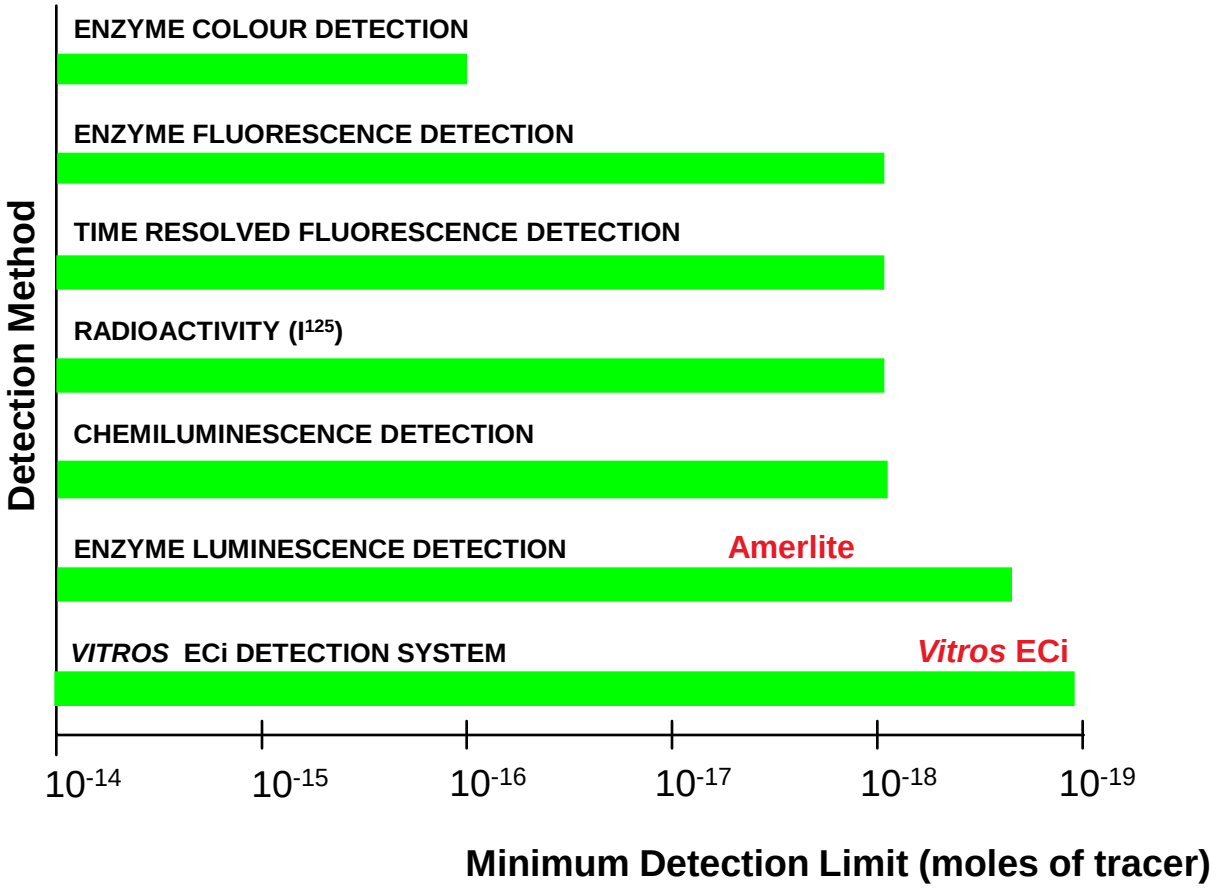
Signal Generation

Comparison in the Sensitivities of Tracers used for Immunoassays



ACMIA : Antibody-conjugated magnetic immunoassay
CEDIA : Cloned enzyme donor immunoassay
CLIA : Chemiluminescence immunoassay
CMIA : Chemiluminescent microparticle immunoassay
ECLIA : Electrochemiluminescence immunoassay
EIA : Enzyme immunoassay
EMIT : Enzyme-multiplied immunoassay technique
FPIA : Fluorescence polarization immunoassay
IA : Immunoassay
LC-MS/MS : Liquid chromatography with tandem mass spectrometry (chromatographie liquide couplée à la spectrométrie de masse en tandem)
LC-UV : liquid chromatography with ultraviolet detection (chromatographie liquide couplée à la détection UV)

MicroImmunoassay Center

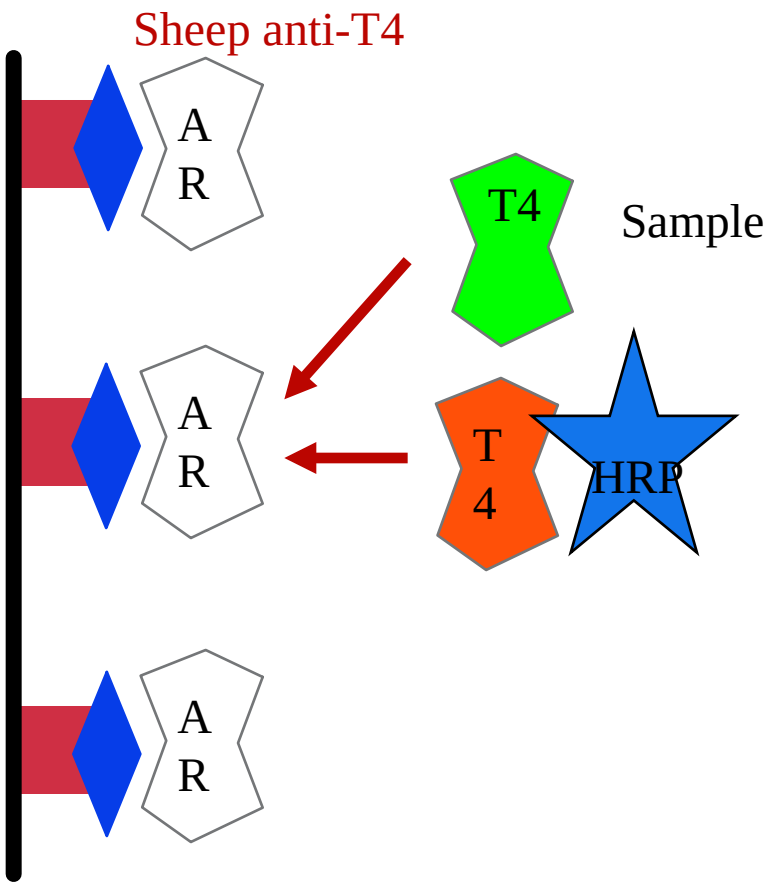
- Integrated Reagent Pack
  - Integrated design
  - Ready-to-use
  - Extended stability
  - Minimizes solid and liquid waste

- Random Access Calibration
  - One to three bar-code labeled calibrator tubes, assay dependent
  - Up to 28-day calibration stability
  - Multiple-lot calibration
  - Automatic Result Protection Calibration

Reduces operator interventions

DAS well - TT4 assay

Vitros Assay step 2



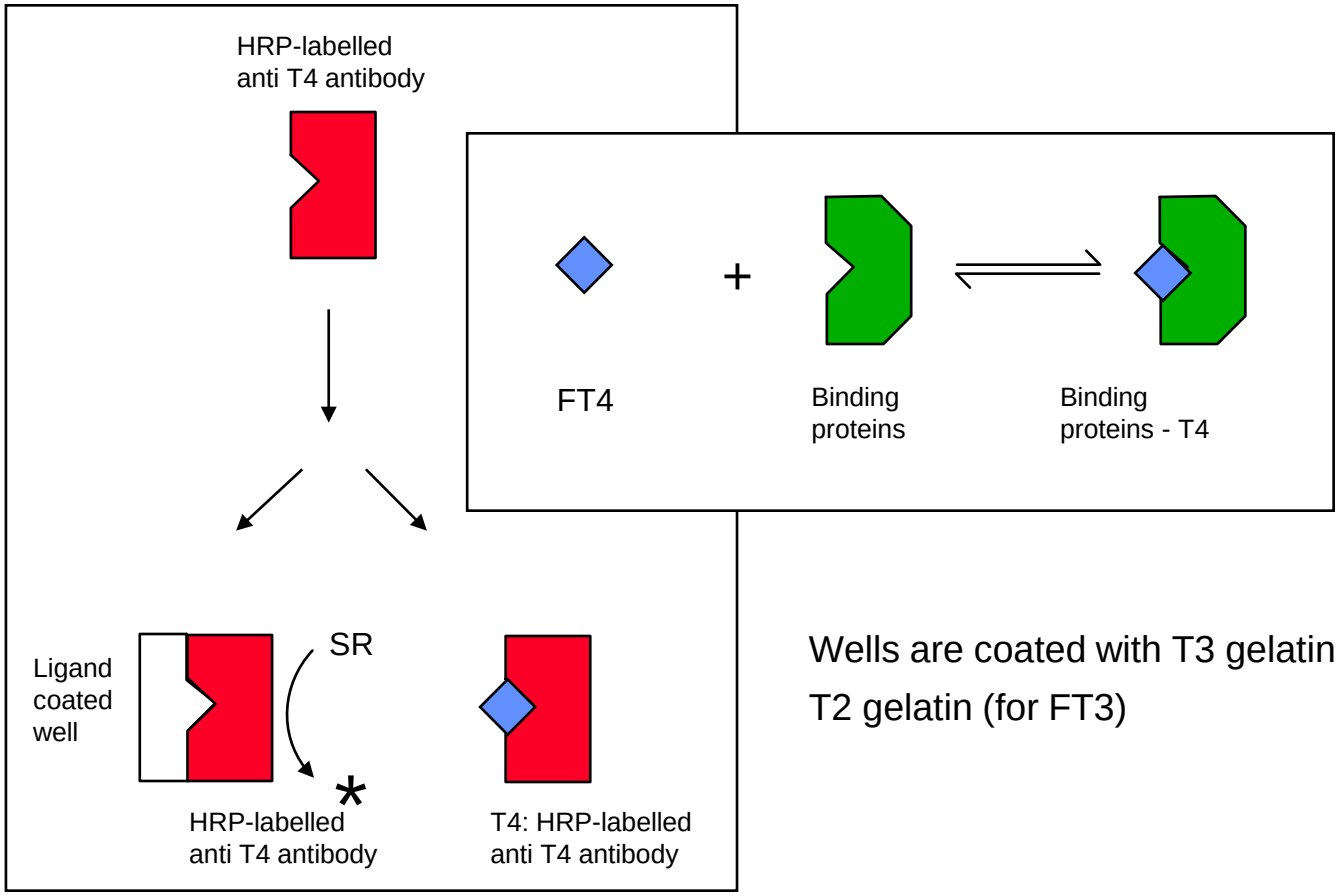
Step 2 - Sample, Assay Reagent and Conjugate added to the wells.

T4 in the sample and labelled T4 in the conjugate compete for the limited number of binding sites available.

FT3 and FT4

The labeled antibody method

Key components: anti-T4 antibody at low concentration and a ligand bound to the well in excess. The labelled antibody can bind T4 with high avidity, but can bind the ligand with low avidity. The ligand in excess captures all antibodies not bound to FT4.



Wells are coated with T3 gelatin (for FT4) or T2 gelatin (for FT3)

Mono and Polyclonal ab: preparation

Polyclonal antibody

- Preparation of an immunogen stimulating the antigenic response

Inoculation of immunogen in suitable mammals (often big, e.g. sheep, donkey, rabbit)

antisera collected contains an heterogeneous mixture of different antibodies

when diluted (at low concentration) only antibodies with highest affinity react

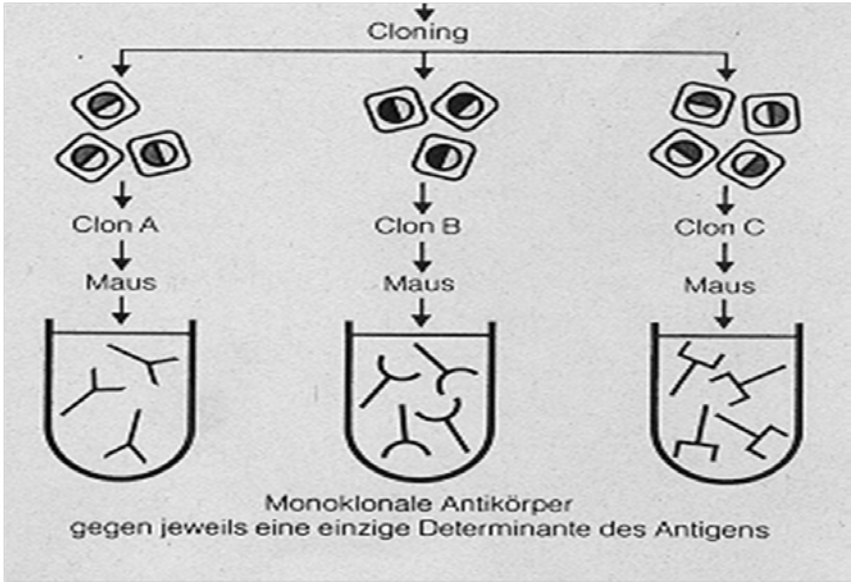
Heterogeneous antisera behave as homogeneous reagent in an immunoassay

Monoclonal antibody

- Inoculation of immunogen in a mouse

After Ab response, the spleen is removed and cells are fused with myeloma cells and grown in cell culture

If the colture produces the desired Ab, it is cloned



Mono and Polyclonal Ab: advantages

Polyclonal antibody

Simple and well established production method

multiple antigenic sites recognition confers high binding propriety

Monoclonal antibody

Monospecificity, even if the immunogen was impure

Affinity defined and can be selected

Clean reagents giving low non specific binding and backgrounds

Indefinite supply of Ab with constant characteristics

Mono and Polyclonal Ab: disadvantages

Polyclonal antibody

- Low specificity, because antibodies bind to a multiplicity of antigenic sites

- Not indefinite supply (animal may die)

Monoclonal antibody

- Low affinity

- Not useful for competitive design

- Poor curve shape (signal vs concentration)

Choosing a Design

When developing new immunoassays, the choice between immunometric and competitive assays depends on :

Analyte: small analytes typically use competitive format (E2)

Specificity: the use of paired monoclonal antibodies can enhance the specificity of immunometric assays, but is less likely to measure all the variable forms of proteins (HBsAg ES)

Sensitivity: while good competitive immunoassays can demonstrate excellent sensitivity, immunometric assays can often improve on this (TropI ES)

Calibration Range: Competitive design is limited because of the slowly diminishing signal which approaches the residual background signal at high concentrations (bHCG II)

So what's in a Reagent?

e.g. Troponin I - A simple Immunometric assay!!!

Biotin Conjugate Reagent

K_2HPO_4 (34.5mM) } pH 6.4
KH_2PO_4 (65.5mM)
Disodium EDTA (15mM)
Heat Treated Horse Serum (10%)
BSA (0.5%)
Proclin 300 (0.5%)
Bovine γ globulin (0.4%)
Triton X-100 (0.1%)
K2 Mab (0.005%)
SCF1 (0.0025%)
Antifoam 204 (20 ppm)
Biotinylated anti cTnI MAb 6.0µg/mL

HRP-Conjugate Reagent

K_2HPO_4 (25mM) } pH 6.4
KH_2PO_4 (75mM)
BSA (0.5%
Proclin 300 (0.5%)
Antifoam 204 (10ppm)
Potassium Ferricyanide (0.001%)
HRP labelled anti cTnI PAb (1.0µg/mL)

Calibrators

Troponin I free plasma
Bovine Gelatin
Liquid BSA
Cyclohexamide (0.002%)
Butylated Hydroxy Toluene

NB exact formulations are not known as base matrix is purchased from a third party.

And don't forget the Water!!

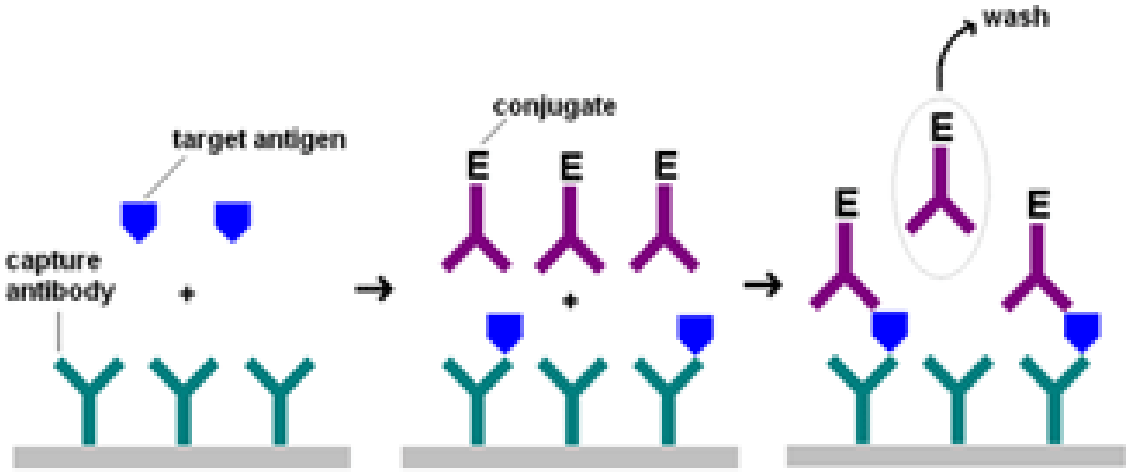
Two Immunoassay Reactions

Immunometric

Two antibodies are used, each binding to a different part of the analyte (antigen) .

One of the antibodies is labeled with HRP enzyme (conjugate)

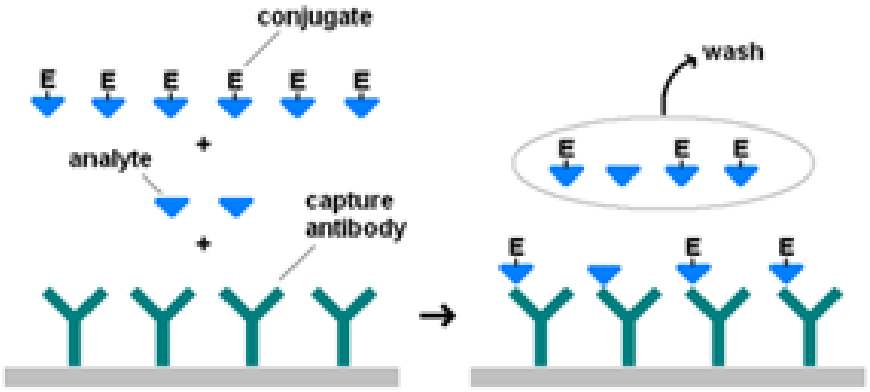
- Antibodies immobilized onto the well are used to capture the analyte present in the sample
- Antibodies labeled with HRP enzyme are added to create an Ab-Ag “sandwich” complex



Two Immunoassay Reactions

Competitive

Unlabeled antigens (analyte) from sample and HRP labeled antigens (conjugate) compete for the antibody immobilized onto the well surface



Two Immunoassay Reactions

The two types of reactions can be in 1 stage or 2 stages

The 2 stages assays have a wash stage prior to the addition of the conjugate.

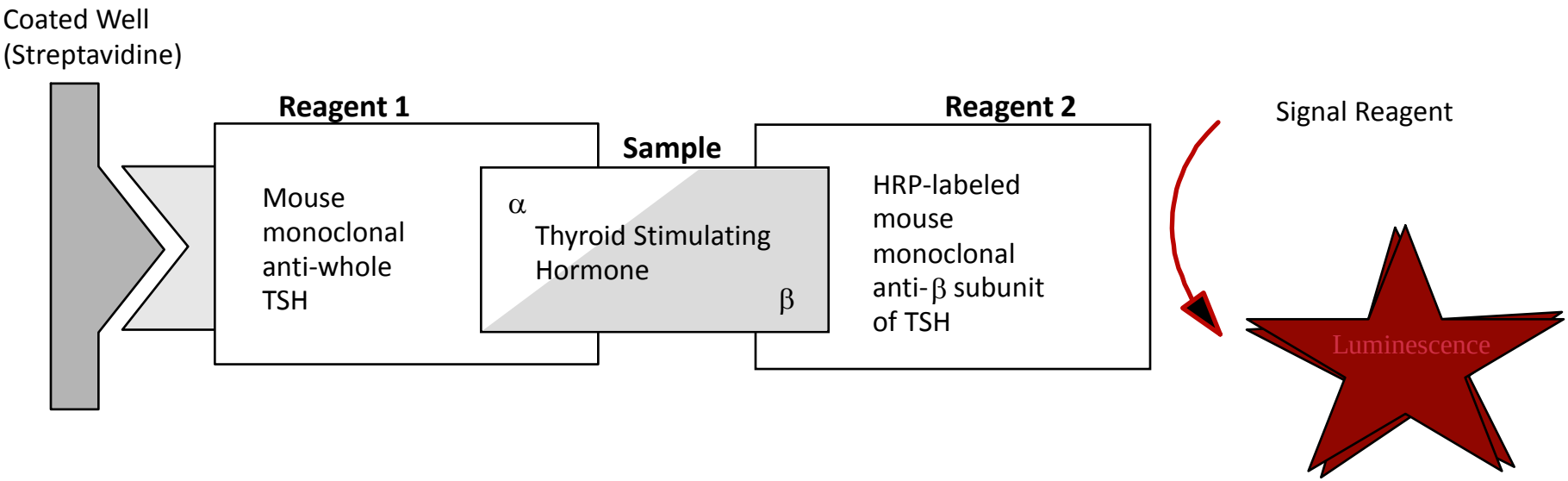
In both types of reaction

the conjugate (labeled with HRP) will react with the luminol to produce blue light

the enhancer amplifies the lasting and the intensity of the signal

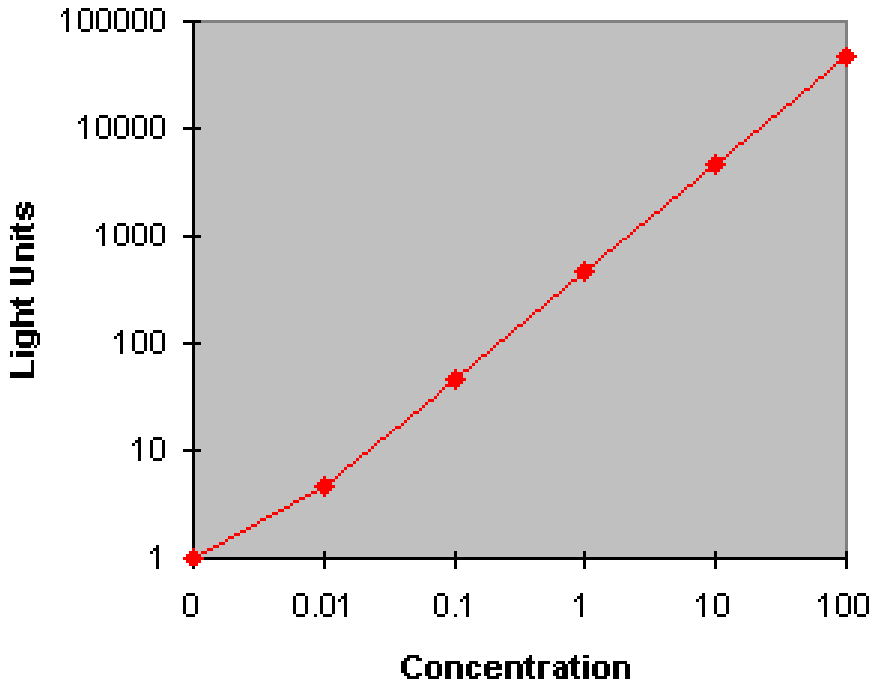
MicroWell™: Immunometric design

Example: TSH



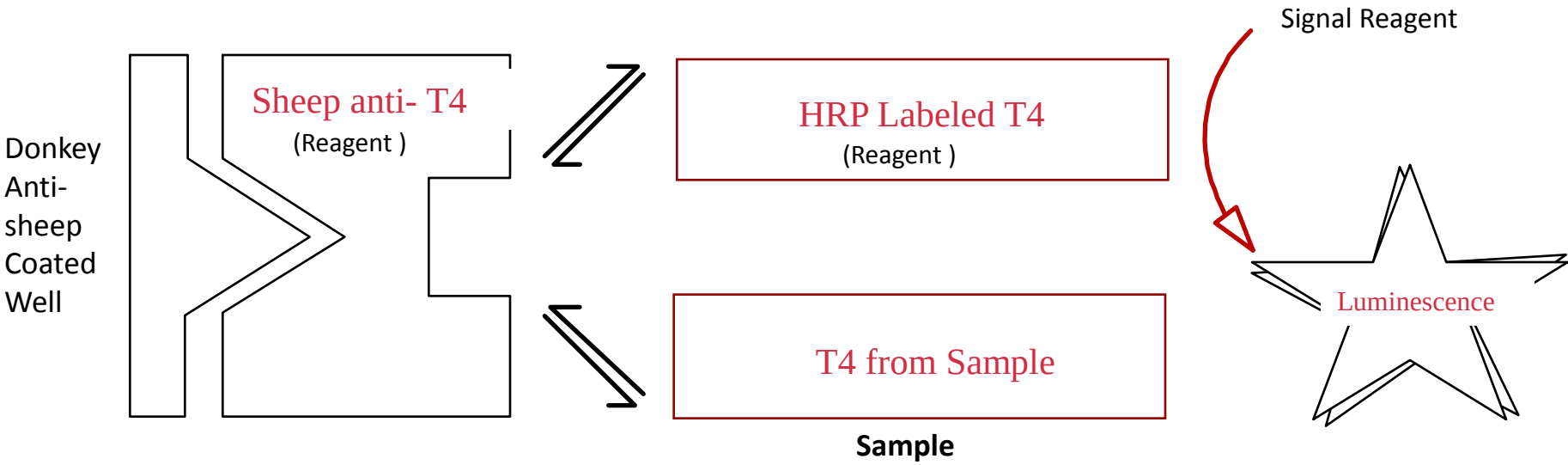
MicroWell™: Immunometric design

The amount of labeled antibody binding and therefore the amount of light will be proportional to the total amount of analyte present in the patient sample



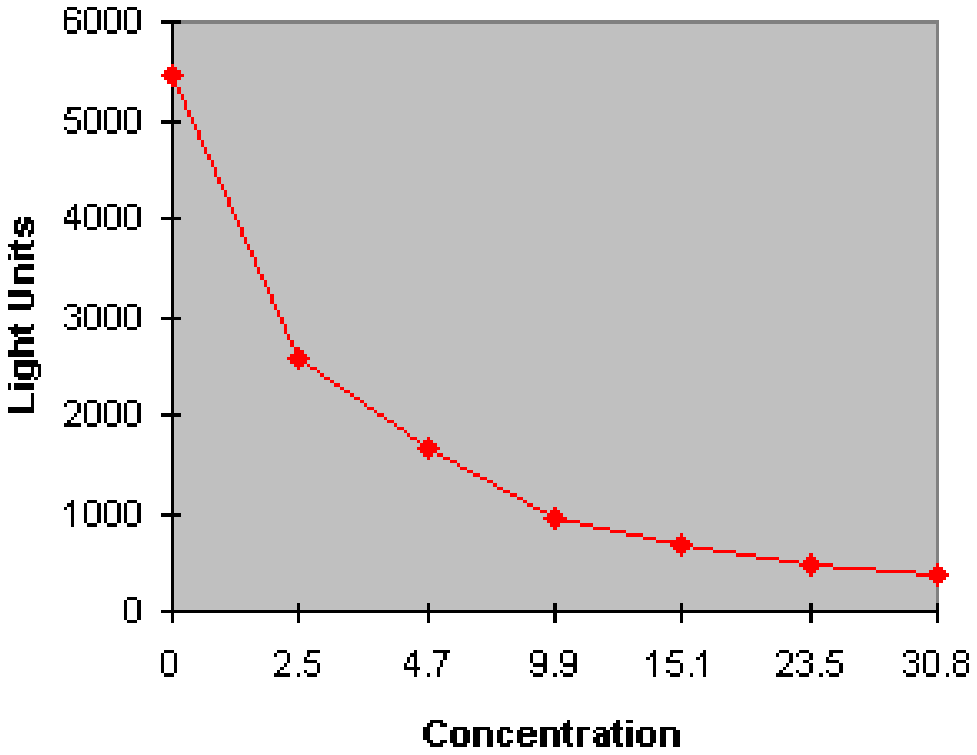
MicroWell™: Competitive Design

Example: TT4



MicroWell™: Competitive Design

The amount of labeled analyte bound to the capture antibody (and therefore the amount of light) is inversely proportional to the concentration of the unlabeled analyte in a patient sample



Immunoassays: quantitative and qualitative

Immunoassays are measuring **small amounts** of biological substances (e.g. can measure 10 at -12 (pico)

Measurements may be:

Quantitative: measuring the actual analyte concentration

Qualitative: testing the presence or absence of a molecule (e.g. reactive or non reactive for antibody). Results expressed as signal/cut-off.

Sensitivity and Specificity

Sensitivity:

the smallest concentration of analyte that can be reliably detected.

- Analytical/ functional sensitivity
- LoB/ LoD
- No false negative result (qualitative assays)

Specificity:

- Ability to measure only what you want to measure
- Sometimes referred as % of cross-reactivity
- No false positive result (qualitative assays)
- Using paired monoclonal antibodies enhances the specificity of immunometric assays (e.g. HBsAg ES)

Sensitivity definitions

Analytical sensitivity :

Lowest concentration at which the assay can differentiate between analyte in a sample and the background noise of the signal measured using a “zero concentration” standard.

Functional sensitivity :

concentration that results in a CV=20% and is thus a measure of an assay’s precision at low analyte levels (precision dose profile)

Sensitivity definitions

Limit of Blank (LoB):

highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested.

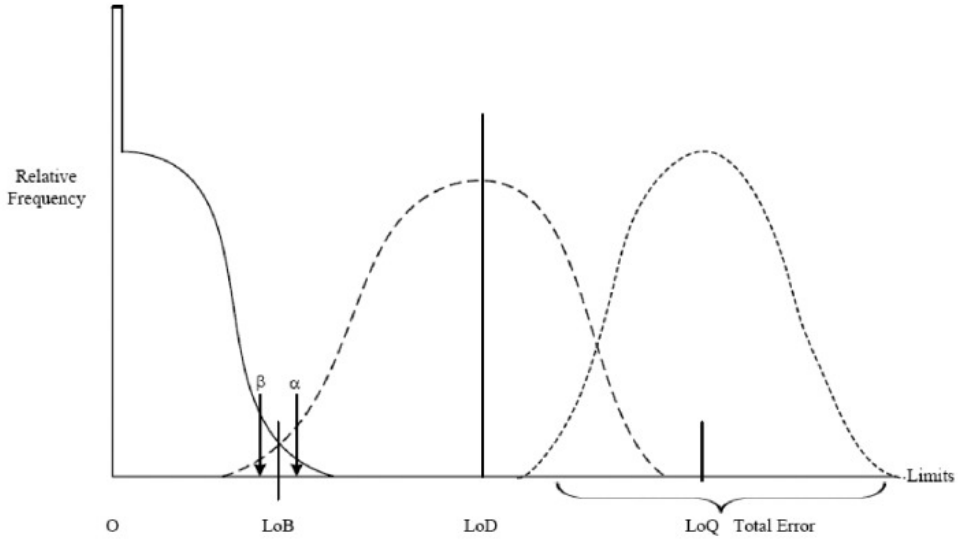
Limit of Detection (LoD):

the lowest analyte concentration likely to be reliably distinguished from the LoB and at which detection is feasible. LoD is determined by utilizing both the measured LoB and test replicates of a sample known to contain a low concentration of analyte.

Limit of Quantitation (LoQ):

the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met.

LoB< LoD ≤ LoQ



Calibration

WHY?

For quantitative tests: Calibration is used to set the mathematical parameters that will be used to calculate the concentrations of an analyte from the responses measured by the analyzer

For qualitative tests: calibration is used to set the signal associated to the Cutoff value

HOW?

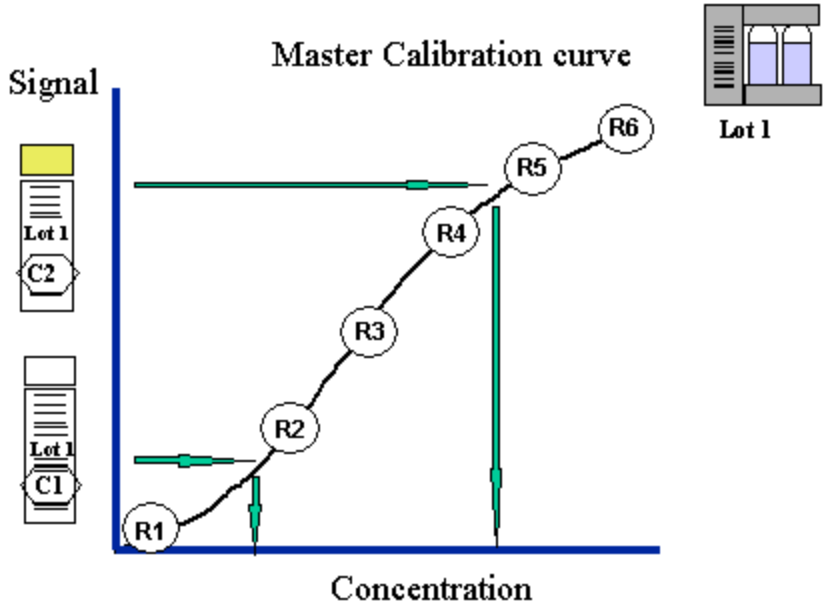
Use fluids with known concentrations: calibrators

System will calculate the mathematical parameters which define the relation between concentrations and responses

Quantitative assays: calibration theory

At manufacturing: Customer calibrators are run on the Master calibration curve.

The calibrators concentrations are determined



TT4 - Serum (nmol/L)

Operator ID:

Reagent Lot: 1010 | Type: User Cal | Date/Time: 6/12/2008 10:43:28

On-Board: No | Status: Current | Kit Lot: 0049

Level/Replicate	Calibrator Value	Response
M1	0. 000	5013. 47
1	3. 29	4722. 00
M2	29. 7	2441. 47
2	60. 3	1504. 00
M3	60. 7	1495. 80
M4	122	884. 922
3	197	606. 000
M5	202	592. 335

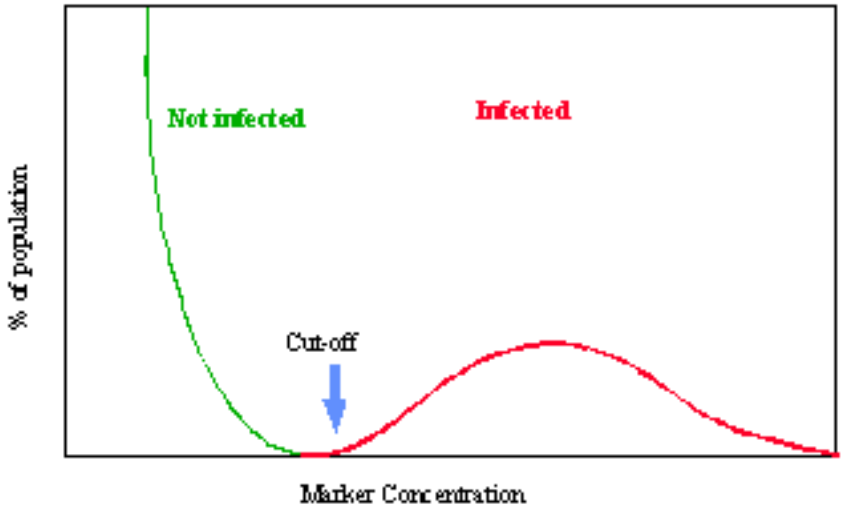
Customer'calibrators concentrations are put on ADD & reagent pack 2D barcode

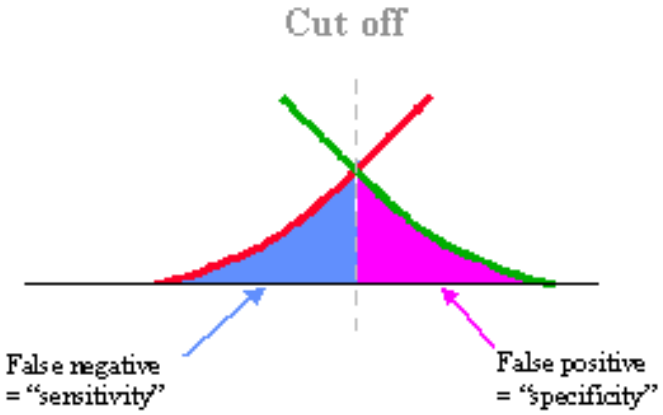
Qualitative assays: calibration theory

At assay development, the cut-off is determined by clinical performance and assay characteristics (remains constant through reagent lots).

The key point for assay performance is to position the cut-off so that specificity and sensitivity are optimized for the proposed application.

Typical clinical performance profile for a quantitative assay:

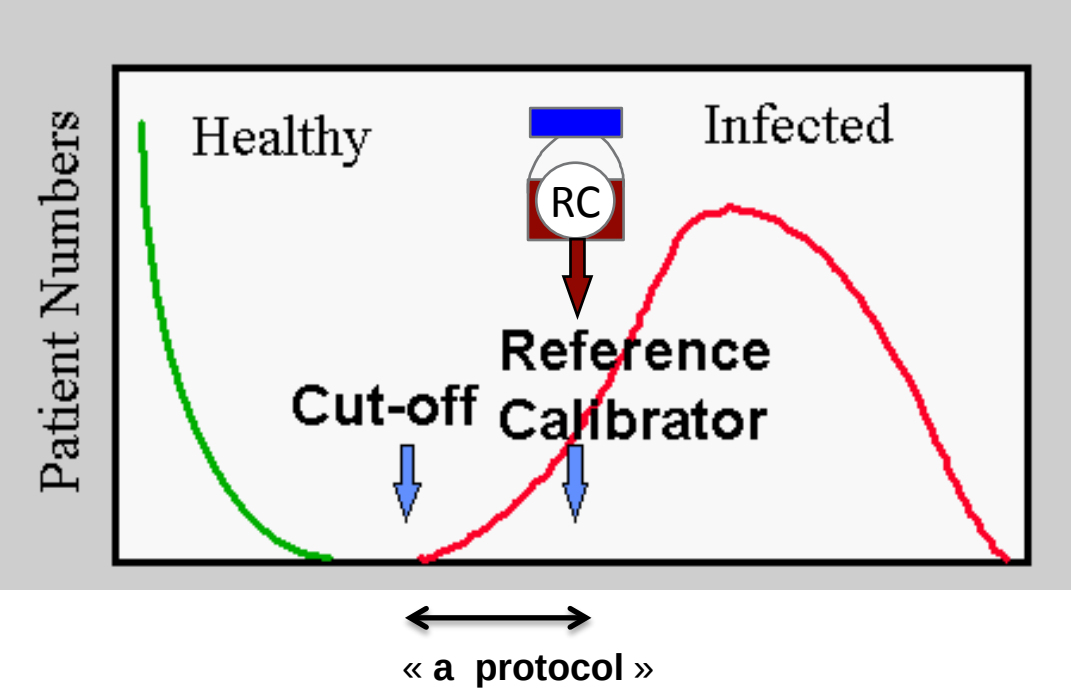




Qualitative assays: calibration theory

The cut-off is fixed by a Reference Calibrator (RC).

Cut-off Signal = RC signal X **a** (protocol)



Heterophile antibodies interference

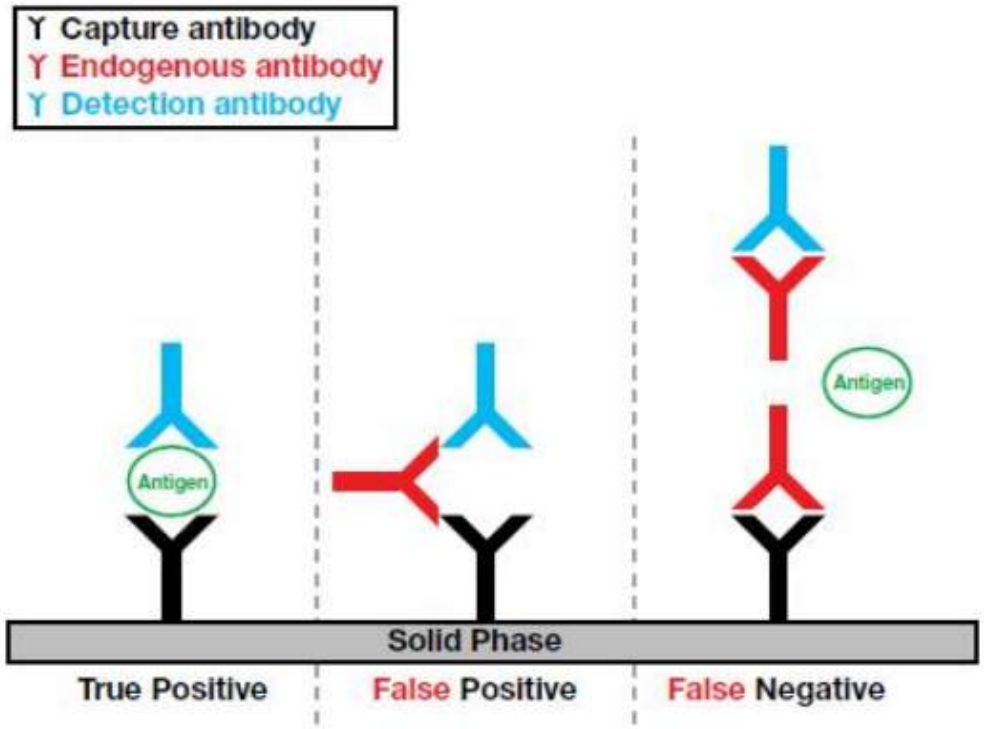
Human anti-animal antibodies (HAAA) are the results of an immunization by using animal derived drugs (e.g. insulin) or vaccines (e.g. HAMA: human anti-mouse antibodies)

Their concentration can persist for days or years.

The prevalence in the population is unknown; a report estimates they are present in 2% of healthy individuals (percentage depends on populations and IA method).

Heterophile antibodies can cause significant interference in any immunoassay. The presence of a heterophile antibody is characterized by broad reactivity with antibodies of other animal species (often the source of the assay antibodies). Human anti-mouse antibodies (HAMA) belong to this category. They can create both false positive and false negative results.

Heterophile mechanism of interference



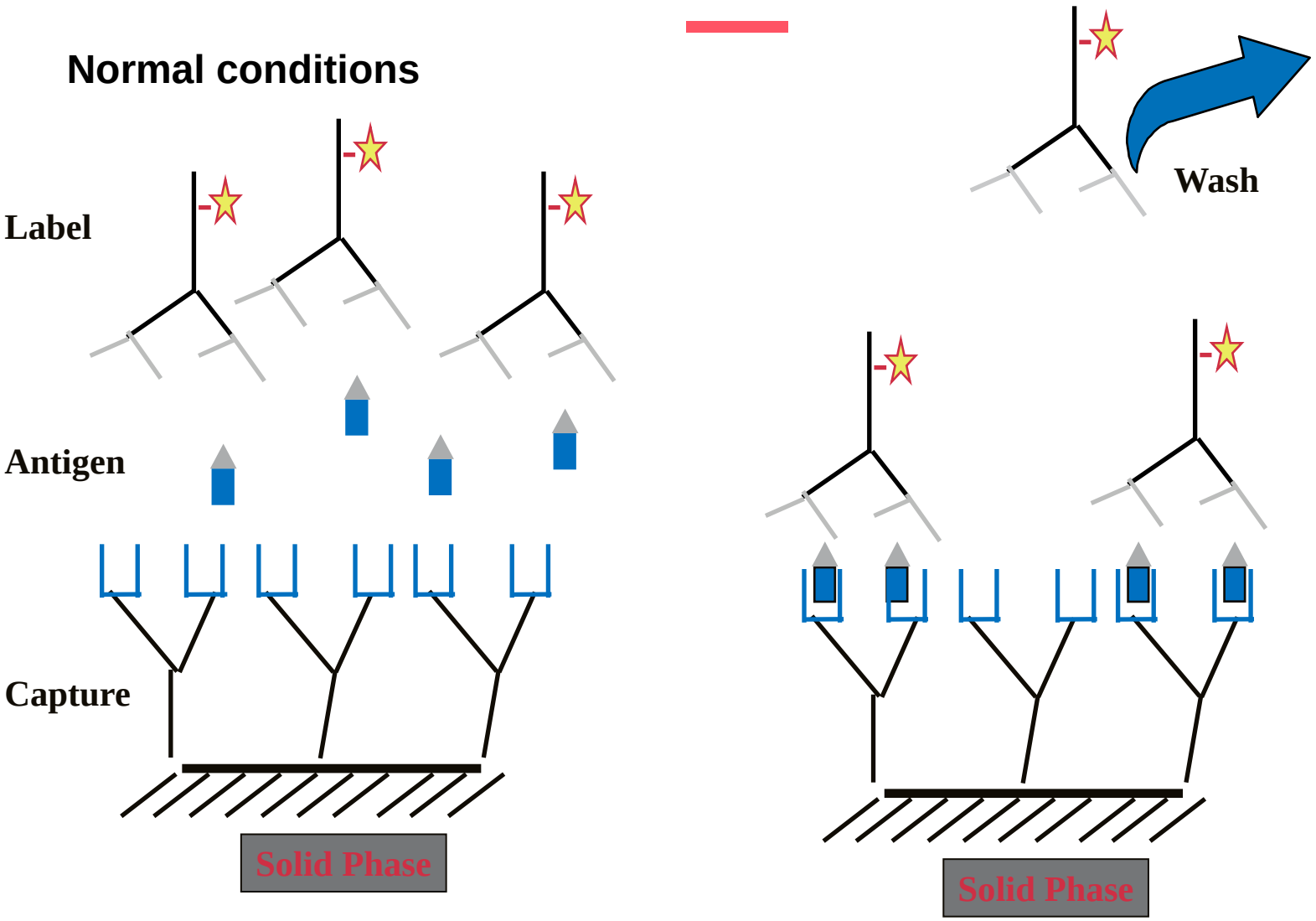
Bridging of capture and detector antibodies
=> Falsely elevated result

Exclusive binding of capture or detector antibody only
=> Falsely lowered result

HAMA interference: solutions

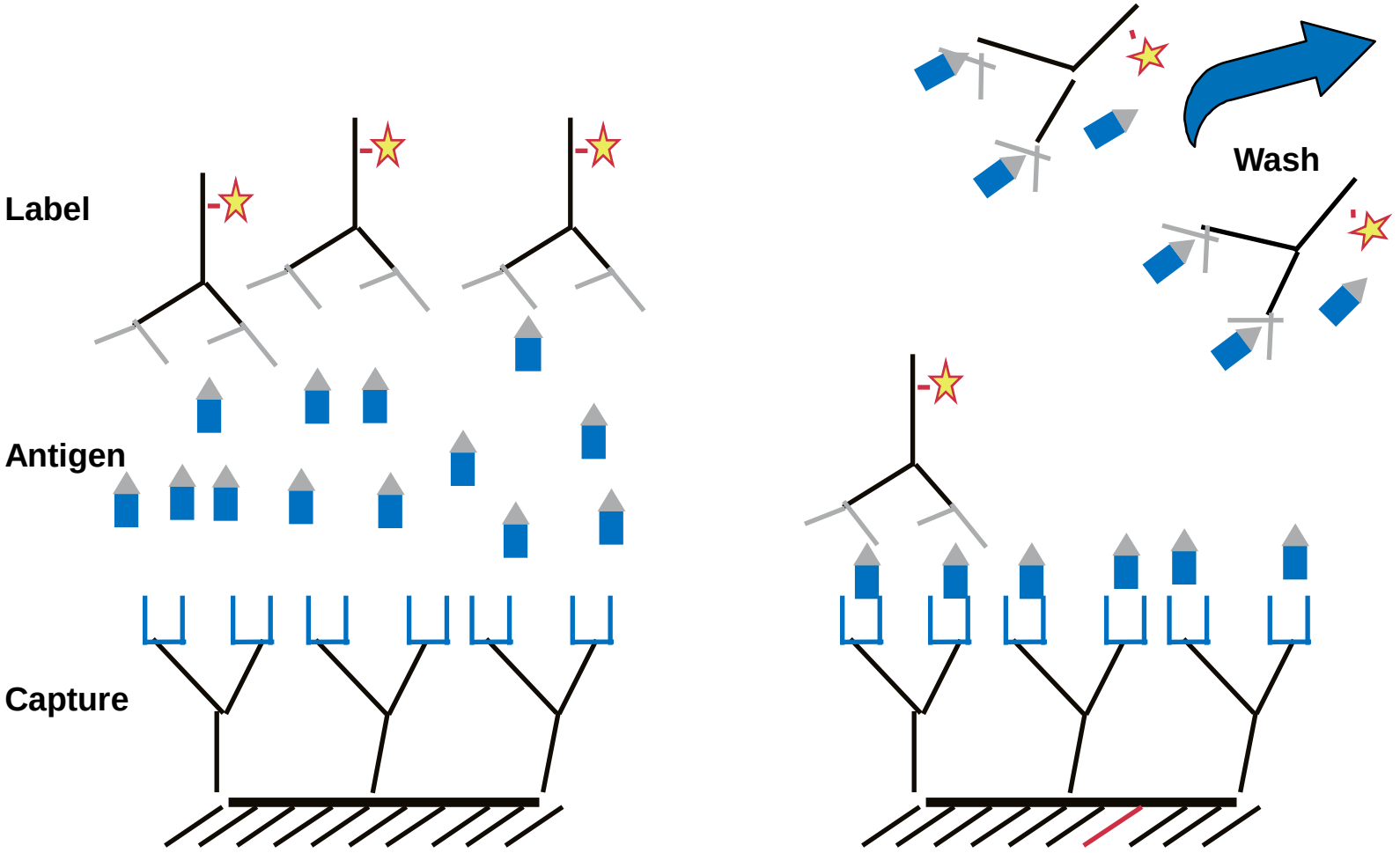
- Repeat the test using a different type of assay, using non-mammalian capture and detection antibodies
- Use of heterophile blocking reagents: most Vitros assays contain **protective factors**, e.g. bovine IgG (see LN d91557). Note: now CEA is protected as well!
- Use **blocking tubes** with binders to inactivate heterophile antibodies:
  - HBT for immunometric assays, detecting antigens (FSH, LH, Prol, TSH, Ferr, CEA, AFP, βHCG, HBsAg, CA 125, CA 19-9). Examples: CEA 32.5 => 0.81 µg/L; TSH 50 => 1.8 µIU/mL)
  - NABT for antidody detection assays (aHCV, aHIV, Toxoplasma, Rubella, CMV). Contain Ig to block non-specific antibodies. Example: HIVc Sample A 115 => 1.14 (NABT), 0.15 (HBT). HIVc Sample B 36 => 1.46 (NABT), 0.23 (HBT).
- **Serial dilutions:** heterophile antibody interference usually doesn't change linearly with serial dilution, but a true result most often will. Therefore non linearity indicates assay interference.

High dose hook effect



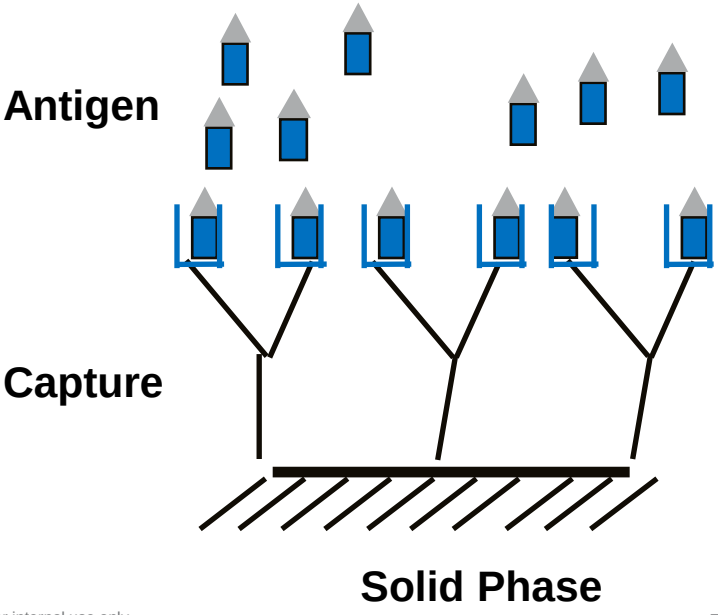
High dose hook effect

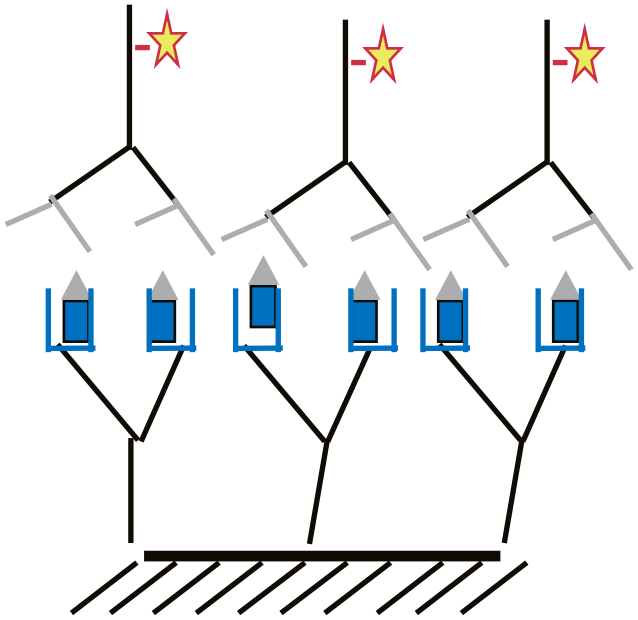
“Hook” conditions



High dose hook effect

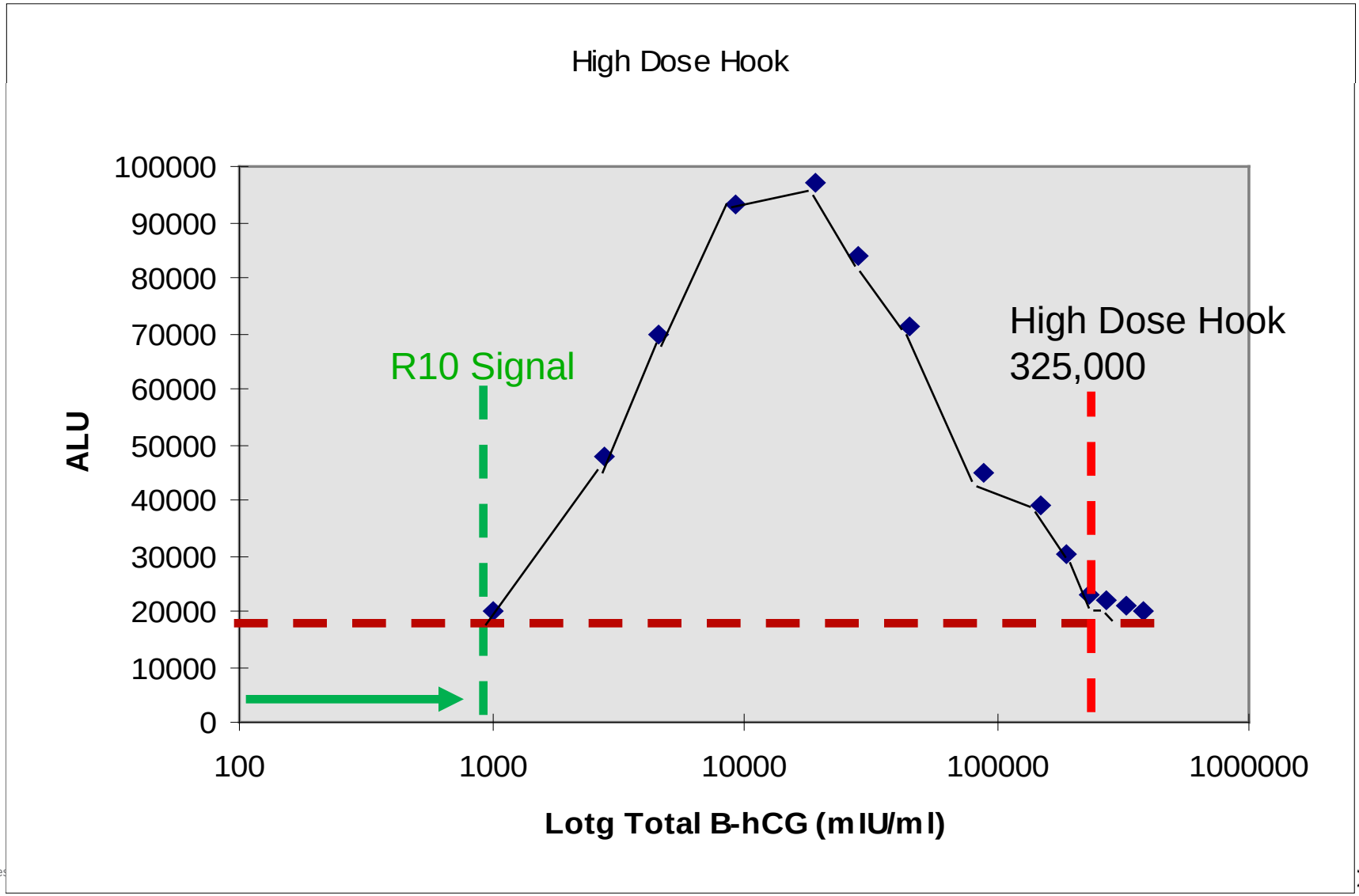
Solving the Problem...





High dose hook effect: total β HCG

New assay: no hook effect in samples up to 1300000 mIU/mL



Vitros Microtip Technology
Facteur rhumatoïd

Ortho Clinical Diagnostics
a Johnson & Johnson company

Features and Benefits of MicroTip™ Technology

- No water or drains
- Eliminates carryover

VITROS VersaTip™ **VITROS MicroTip™**

- Small sample size
- Minimal liquid waste

MicroTip Assays menu

DAT's

Amphetamins
Barbiturates
Benzodiazpines
Phenyclidine
Cannabinoides
Cocaine metabolite
Opiace
Metadone

TDM's

Gentamicin
Tobramycin
Valproic Acid
Vancomycin
Caffeine

Specialty

Direct LDL
Direct %A1c
Rheumatoid Factor
hsCRP
Direct TIBC
Homocystein

Proteins

Transferrin
IgG
IgA
IgM
Microalbumin
ApoA1
ApoB
C3
C4
Prealbumin
ASO
AAT
HPT

MicroTip™ Chemistries Methodologies Overview

Colorimetric Assay:

dLDL

Turbimetric Immunoassay:

All proteins, except from hsCRP and RF

Latex Enhanced Turbidimetric Immunoassay:

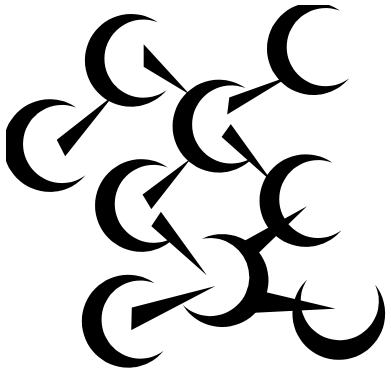
hsCRP and **RF**

Turbimetric Inhibition Immunoassay (TINIA):

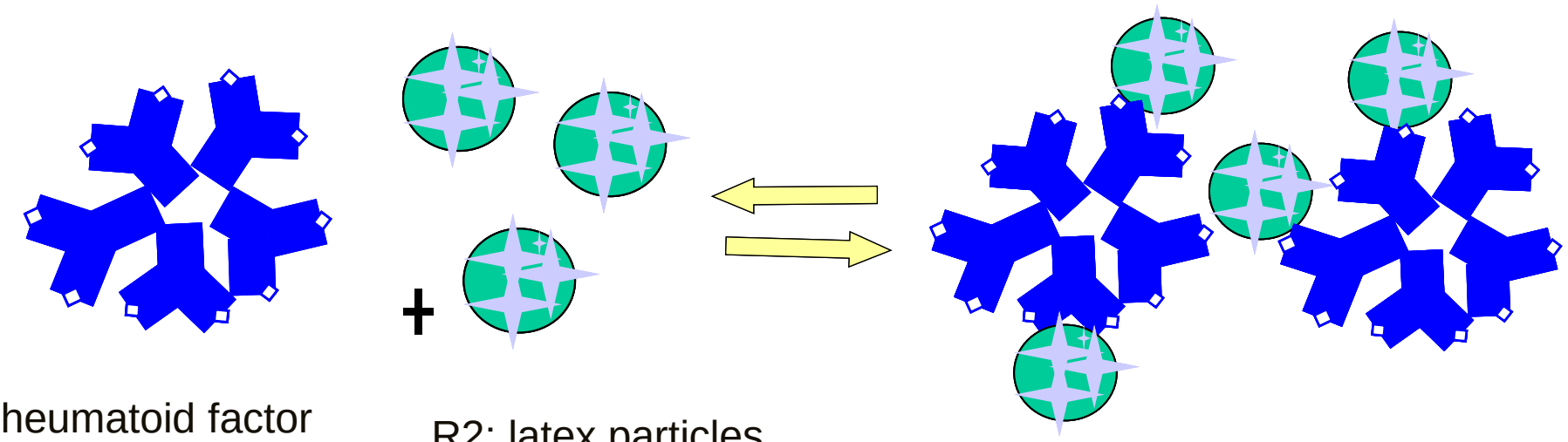
d%A1C

Enzyme Multiplied Immunoassay (EMIT):

TDMs & DATs



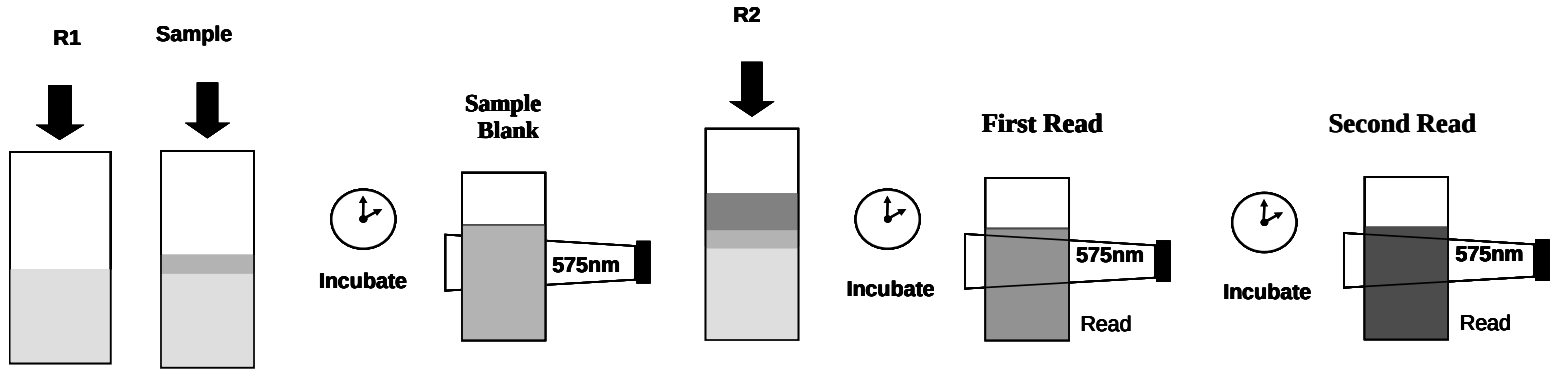
RF : Latex Enhanced Immunoturbidimetric Assay



Rheumatoid factor predominantly IgM anti-IgG antibodies

R2: latex particles adsorbed with denatured human IgG

RF: Latex Enhanced Turbimetric Immunoassay
2 Point Rate protocol



Chem	Dilution	R1ul	Sample(ul)	R2(ul)	Wavelenght
RF	Neat	120	5	40	575nm

Résumé et principe du dosage

Le facteur rhumatoïde (RF) est constitué d'autoanticorps immunoglobulines d'isotypes IgM, IgA, IgG et IgE [1]. La fonction du RF reste peu claire, mais il semble jouer un rôle dans la régulation de l'immunité humorale et cellulaire et dans la protection contre l'invasion de microorganismes [2]. La plupart des patients atteints de polyarthrite rhumatoïde et du syndrome de Sjögren présentent des taux élevés de RF. Le RF peut également être élevé dans la sclérodermie, la dermatomyosite, la maladie de Waldenström, la sarcoïdose et le lupus érythémateux systémique [3]. On a également observé des taux élevés de RF sans maladie apparente ni troubles cliniques définis [2].

Principe de la méthode

Le dosage quantitatif du facteur rhumatoïde est réalisé avec le réactif VITROS Chemistry Products RF en association avec les jeux d'échantillons de calibrage VITROS Chemistry Products Calibrator Kit 16 et FS Calibrator 1 sur les systèmes de chimie clinique VITROS 5,1 FS/4600 et systèmes intégrés VITROS 5600.

Le réactif VITROS RF est une cartouche à double compartiment contenant des réactifs liquides stables et prêts à l'emploi, utilisés dans une réaction en deux temps pour la mesure quantitative du facteur rhumatoïde. Dans un premier temps, l'échantillon contenant le facteur rhumatoïde est dilué dans le tampon contenu dans le réactif 1. Une réaction antigène-anticorps se produit dans un deuxième temps, entre le facteur rhumatoïde de l'échantillon et les IgG humaines dénaturées adsorbées sur les particules de latex du réactif 2, produisant une agglutination. L'agglutination est détectée par un changement d'absorbance à 575 nm, l'importance de la variation étant proportionnelle à la quantité de RF dans l'échantillon. Une fois le calibrage effectué pour chaque lot de réactifs, la concentration en RF de chaque échantillon à tester peut être calculée à l'aide de la courbe d'étalonnage mémorisée et de l'absorbance mesurée obtenue lors du dosage de l'échantillon.

Type de test et conditions d'exécution

Type de test	Système VITROS	Durée approximative d'incubation	Température	Longueur d'onde	Volume de la goutte d'échantillon
Dosage cinétique en deux points	5600, 4600, 5,1 FS	Incubation 1 : 5,1 minutes Incubation 2 : 1,5 minutes	37 °C	575 nm	5,0 µL

Gamme de mesures (linéarité)

Unités conventionnelles (UI/mL)	Unités SI (kUI/L)
8,6–120,0	8,6–120,0

Traçabilité de l'étalonnage

Les valeurs attribuées aux jeux d'échantillons de calibrage VITROS Chemistry Products Calibrator Kit 16 et FS Calibrator Kit 1 pour le dosage du facteur rhumatoïde sont dérivées de la *Préparation de Référence Internationale de Sérum de Polyarthrite Rhumatoïde*, OMS 1ère Norme Britannique, NIBSC 64/2 [9].

Valeurs attendues

Valeurs de référence

L'intervalle de référence est défini comme les 97,5% des résultats d'une étude réalisée sur 507 adultes apparemment sains.

Unités conventionnelles (UI/mL)	Unités SI (kUI/L)
< 12	< 12

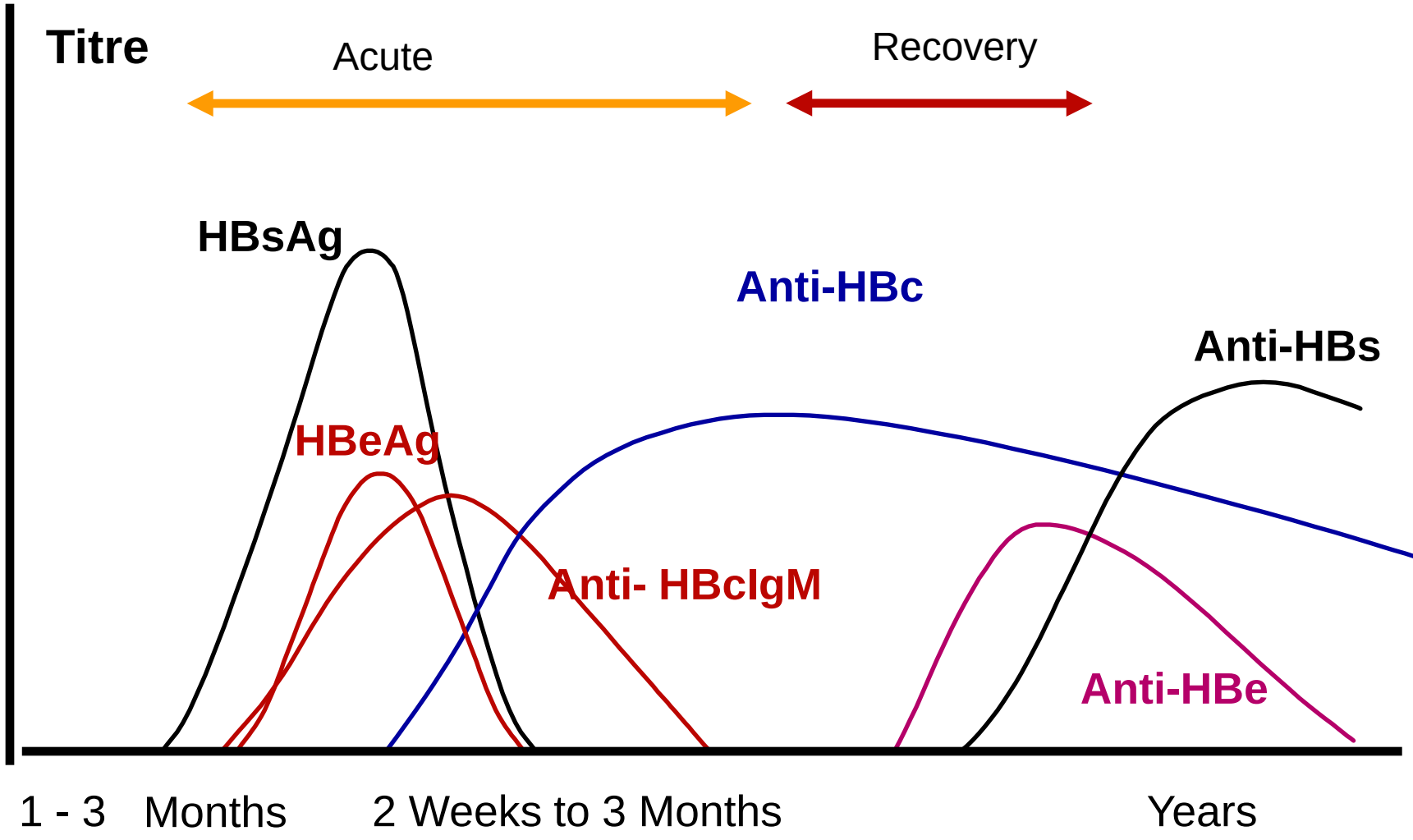
Chaque laboratoire doit confirmer la validité de cet intervalle sur sa propre population.

Ortho Clinical Diagnostics

Immunoassay portfolio

- **Infectious diseases** (HBV, HAV, HCV, HIV, Syphilis)
- **Thyroid** (TSH, FT3, FT4, TT3, TT4)
- **Cardiology** (TropI ES, Myoglobin, CKMB, NTproBNP)
- **Reproductive Endocrinology** (Prog, FSH, LH, Testo, tβHCG II, Prol, E2)
- **ToRC** (Toxo IgM, Toxo IgG, Rub IgM, Rub IgG, CMV IgM, CMV IgG)
- **Anemia** (Ferr, Vit B12, Folate)
- **Oncology** (tPSA II, freePSA, CA 19-9, CA 15-3, CA 125, CEA, AFP)
- **Metabolism and bone** (Vit D, NTx, CORT, iPTH)
- **Renal** (NephroCheck)
- **Diabetes** (Insulin, C-Peptide)

Acute Hepatitis B infection



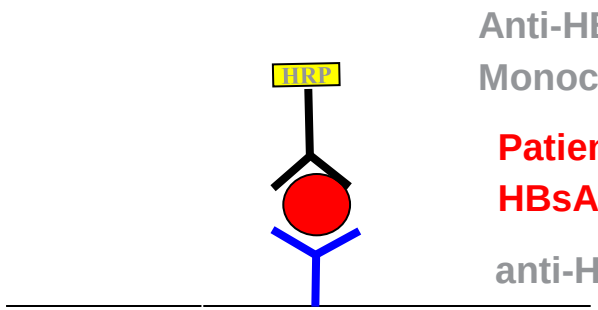
VITROS HBsAg / HBsAg ES Assay Comparison

“Immunometric” Assay Format
Anti-HBs coated well
80 µl sample
50 µl assay reagent
20 µl conjugate reagent
Incubate 29 minutes
Wash
200 µl SR
Read

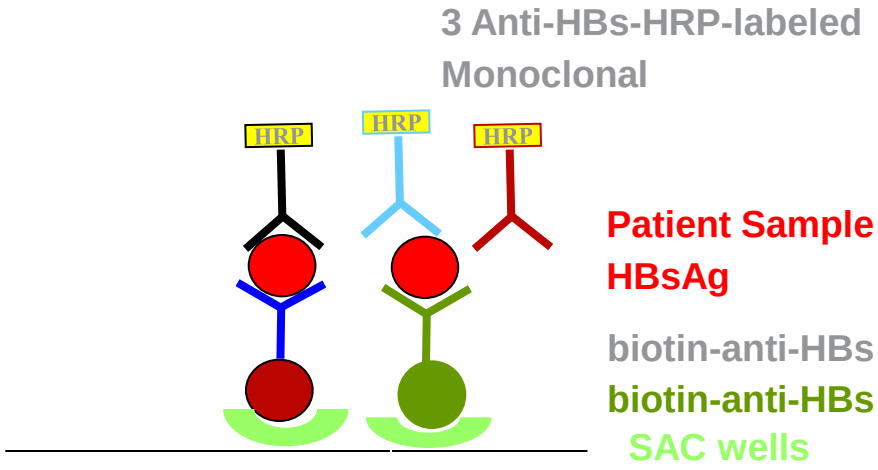
SAC-well
80 µl sample
50 µl biotin reagent
20 µl conjugate reagent
Incubate 29 minutes (single step)
Wash
200 µl SR
Read

37 minutes time to first result

HBsAg assay



HBsAg ES



Determinant a

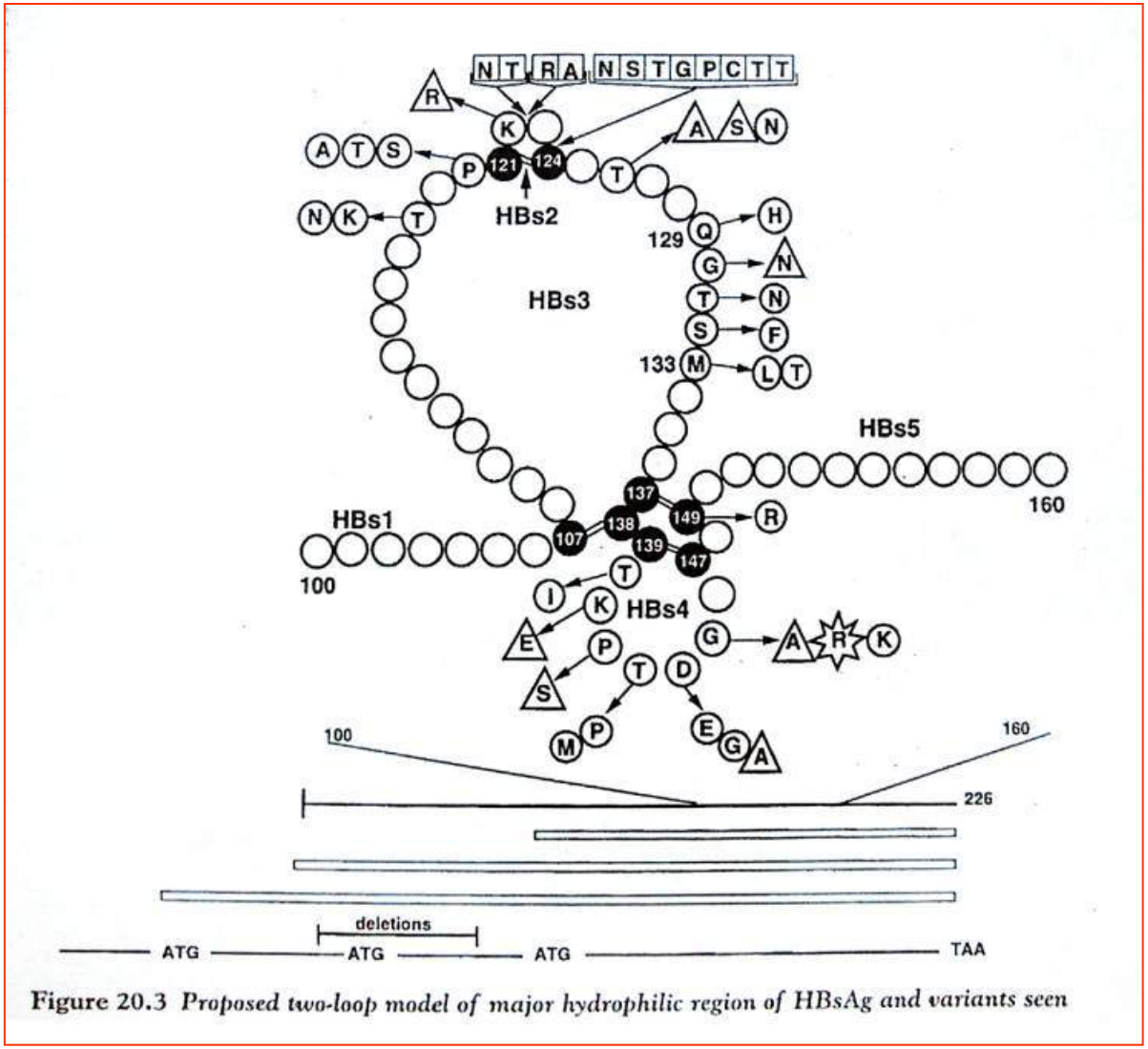


Figure 20.3 *Proposed two-loop model of major hydrophilic region of HBsAg and variants seen*

DEVELOPMENT OF A NEW HBsAg ASSAY WITH IMPROVED SENSITIVITY TO WILD TYPE AND VARIANT HBsAg FOR USE ON THE ORTHO CLINICAL DIAGNOSTICS VITROS® ECiQ IMMUNODIAGNOSTIC SYSTEM.

Van Cleve[2] M., Son S[2]., Todd H[2]., Ching C[2]., Herring B[2]., Zheng J[1]., Kilmartin P[1].

Ortho-Clinical Diagnostics[1] and Chiron Corporation[2] . [1] Rochester, NY and [2]4560 Horton St Emeryville CA 94608

Objectives: The need to improve the detection of HBsAg to overcome the effect of variants and mutants, as well as increase general sensitivity, is evident from literature. The objective was to increase the ability of the VITROS assay to detect a range of HBsAg variants and to further increase the sensitivity of the test to wild type HBsAg.

Materials and Methods: 60 monoclonal antibodies were characterized on their binding patterns of mutant HBsAg and wild type antigen. **Improvements were assessed for detection of seroconversion panels and enhanced analytical sensitivity for wild type, subtypes and recombinant mutants.** A range of artificial recombinant mutants was generated from the first loop (124-137) and second loop (137-147) regions that have the greatest effect on conformation. Tests employed a standard VITROS® ECiQ System with Intellicheck™.

Conclusions: **For HBsAg mutants in or around the first loop with recombinant materials the sensitivity was improved by 11%-100%.** For the second loop variants there was an increase in sensitivity for all mutants, average of 0.14 ng/ml. All seroconversion panels could be detected at the same or an earlier time-point compared with many commercially available HBsAg assays. Specificity performance on serum is excellent.

"Dr. Echevarria" Study –Performed in Spain with naturally occurring HBsAg mutations

Journal of Medical Virology 80:598–602 (2008)

Improved Detection of Natural Hepatitis B Virus Surface Antigen (HBsAg) Mutants by a New Version of the VITROS® HBsAg Assay

José M. Echevarría* and Ana Avellón
Service of Diagnostic Microbiology, National Centre for Microbiology, Instituto de Salud Carlos III. Majadahonda, Madrid, Spain

Supports Key Sensitivity Claim to HBsAg Mutations

…In a study of HBsAg positive human samples containing naturally occurring single and multiple amino acid substitutions across the a-determinant region of HBsAg, 67/67 were found to be reactive in the VITROS HBsAg ES assay

Hepatitis B Surface Antigen Mutants on Five Immunoassay Analysers

S Saw, B Saw and S Sethi

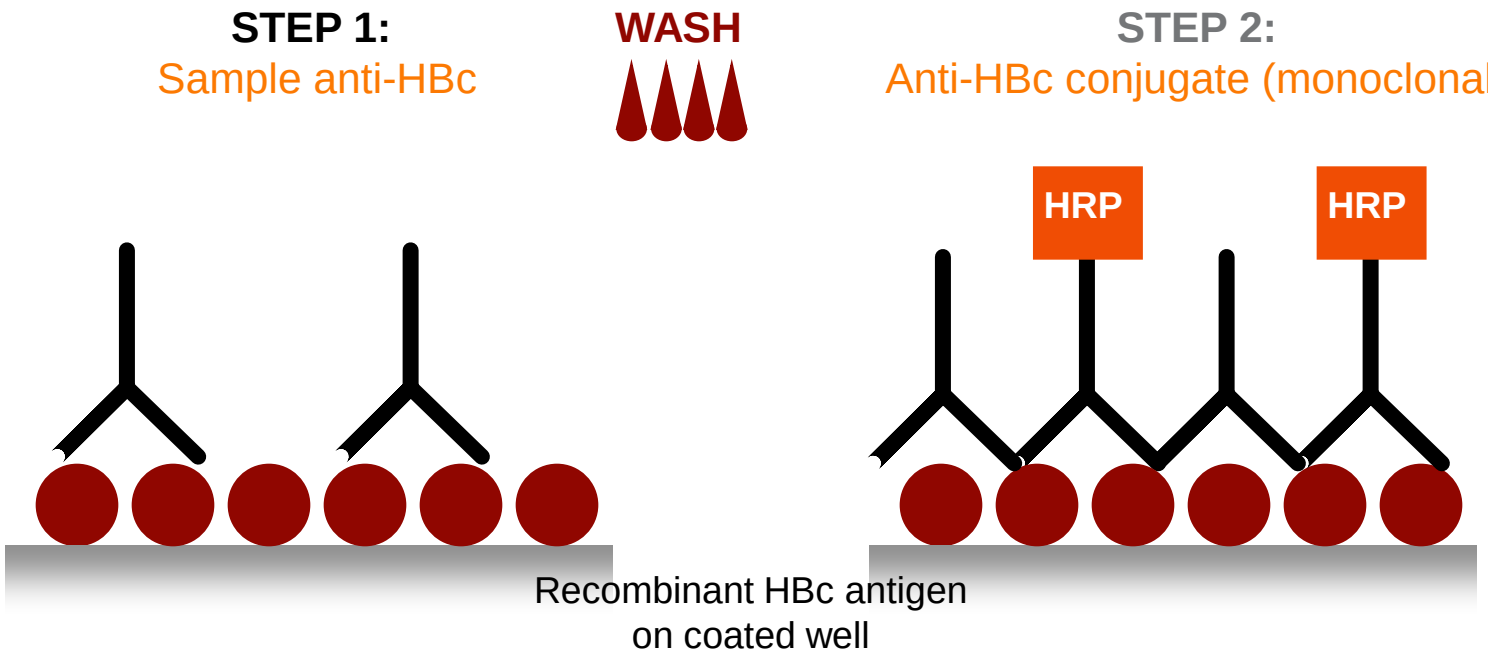
Department of Laboratory Medicine, National University Hospital, Singapore

Mutation at position	Elecsys [COI]	Centaur [Index]	Immulite2000 [S/CO]	ECI [COI]	Axsym [S/CO]
F8L/R24K/N40R/G43R/L94S/ M103I/I13A114/M133T/P142L/D144G	5.92	<0.10	2.13	1.17	3.12
I110L/S113T/T114S/T126I/ N131T/F134Y/T143S/G145R	5.17	<0.10	1.45	1.01	3.30
S132Y/P142S/G145R	12.01	<0.10	4.02	1.14	9.95
Q129P/F134R/P142L/ D144E/G145K/S171F/L175S	4.92	<0.10	0.650	1.03	2.11
R122I	2.92	0.31	0.810	2.20	1.43
R122T	10.78	2.55	0.740	28.0	4.59
C124R	5.25	<0.10	0.575	2.58	0.94
E122I	3.83	2.04	0.962	1.45	1.36
T123N	69.64	14.95	54.0	106	0.63
G145K	2.16	<0.10	2.70	1.01	2.32
122RA123	4.27	0.38	0.630	1.82	0.68
P142L/G145R	1.78	<0.10	4.54	0.96	2.86
D144G	5.2	<0.10	2.40	3.55	1.64

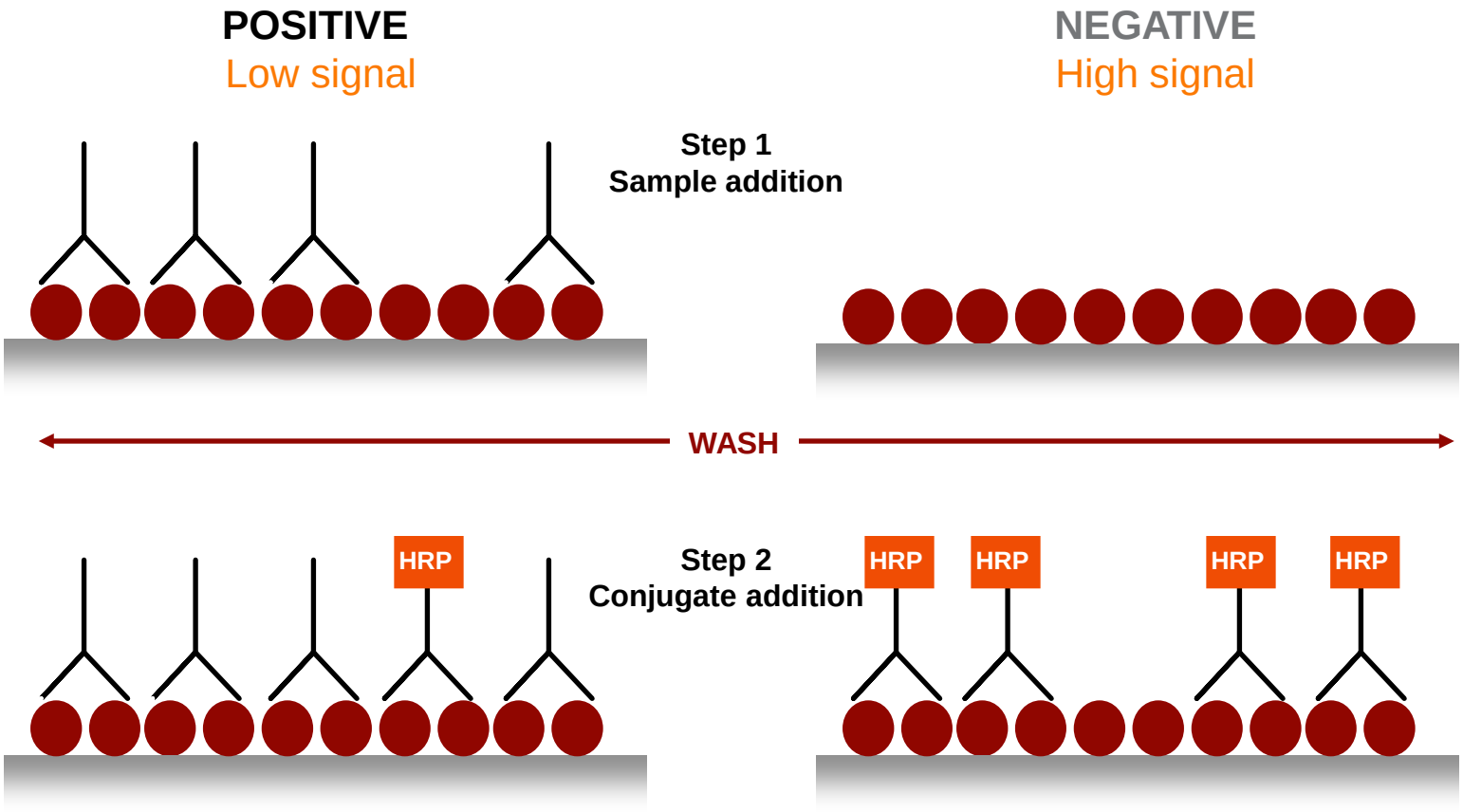
Anti HBc

Assay Design

- Two-step
- Semi-quantitative
- Sequential competitive

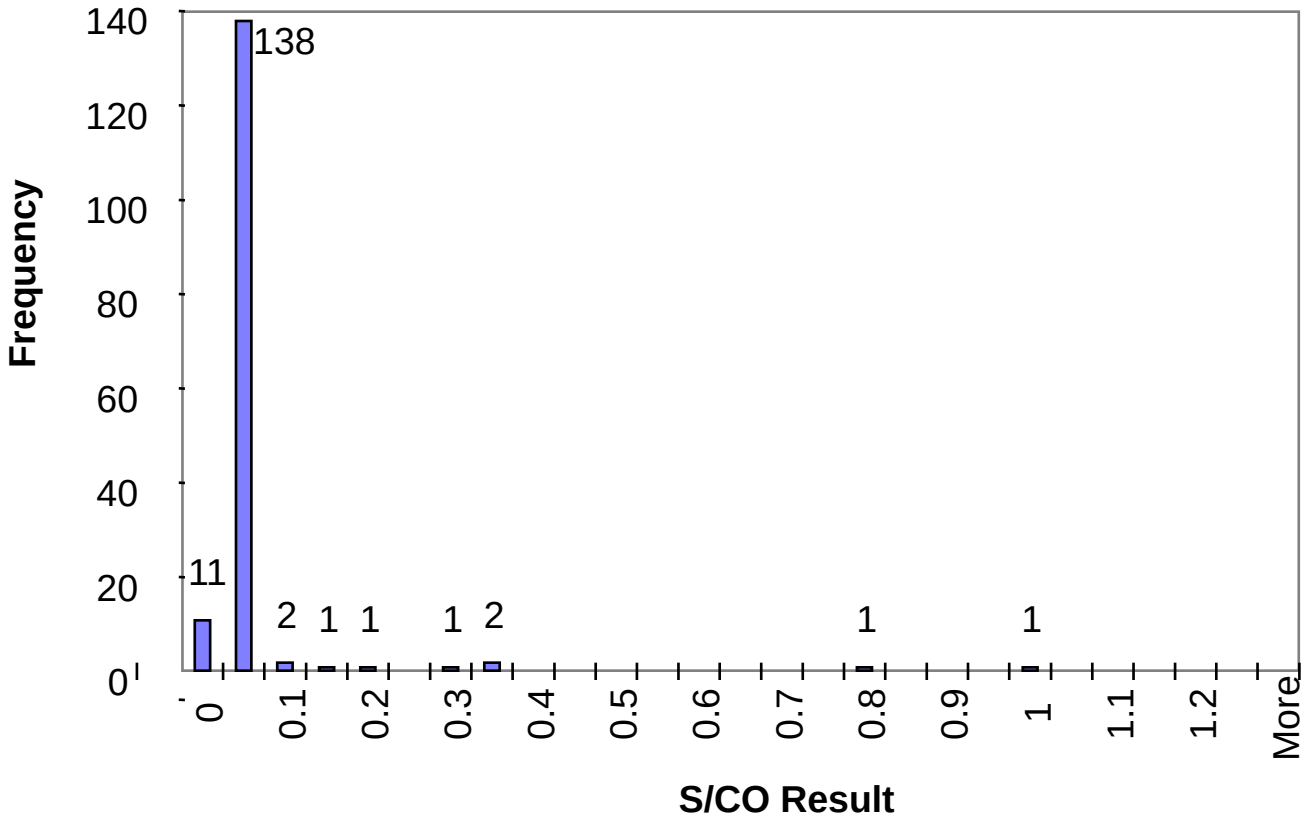


Sequential competition

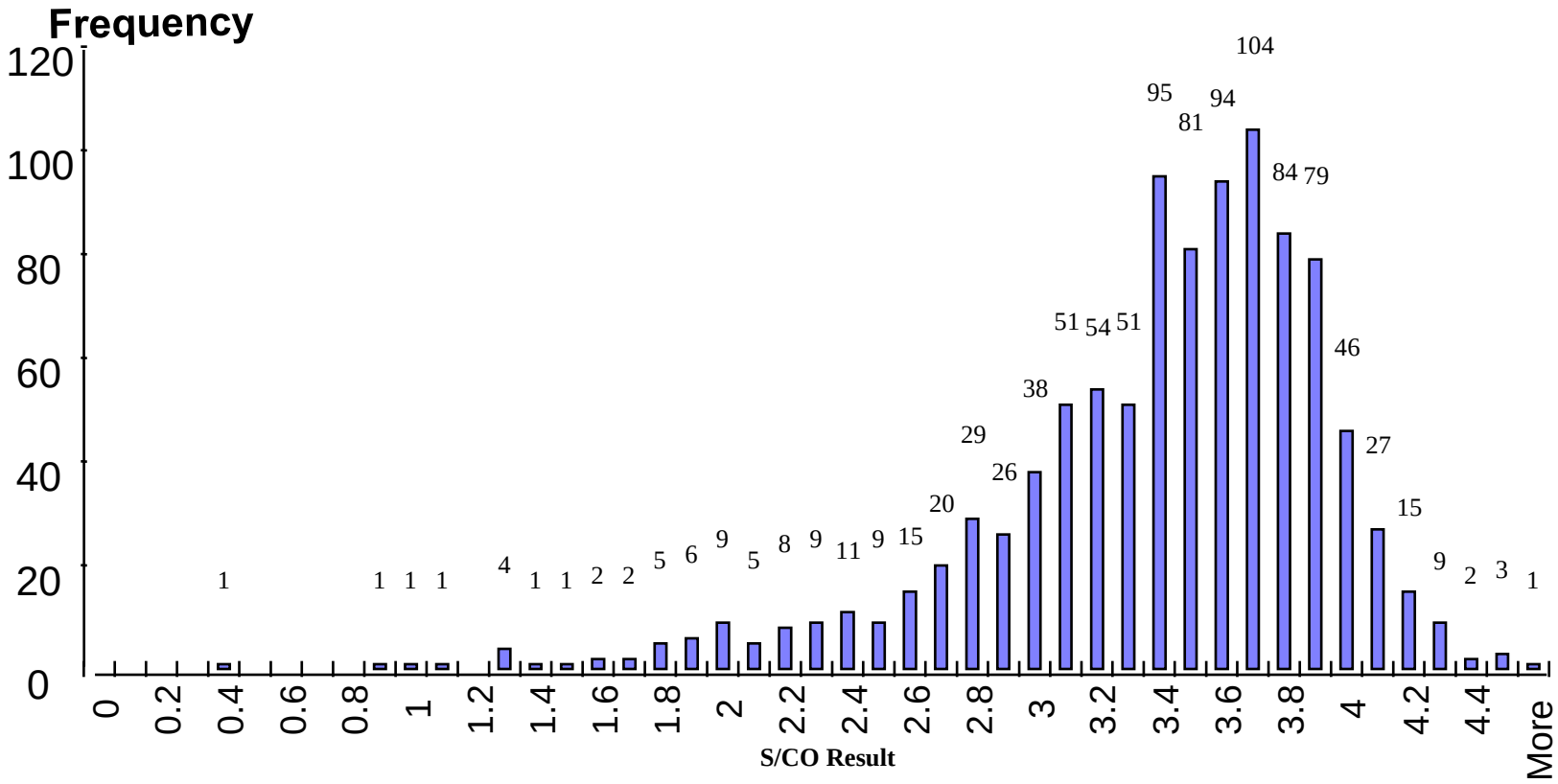


Positive sample distribution

A result of <1.00 indicates a reactive sample and the presence of anti-HBc.
A result of ≥1.00 and <1.20 indicates a borderline sample.
A result of ≥1.20 and <4.80 indicates a non-reactive sample, negative for anti-HBc.
A result of ≥4.80 indicates a sample that requires dilution and re-test.



Negative sample distribution



(n=1000). Specificity = 99.6%

HBeAg/
Anti- HBe

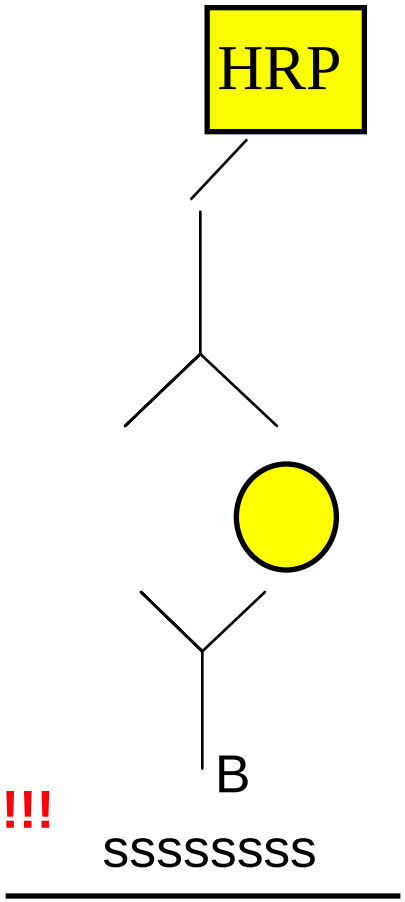
Vitros HBeAg assay

- Single Step
- Immunometric
- Semi-Quantitative
- TTFR 35 minutes

Result classification:

<0.8	Negative
≥ 1.2	Reactive
≥ 0.8 to <1.2	Borderline

Centrifugation (=HBsAg)!!!



Anti-HBe~HRP conjugate (monoclonal)

Sample HBeAg

Biotinylated Anti-HBe (monoclonal)

Streptavidin coated well

Comparative Seroconversion sensitivity Profile Diagnostics. Panel RP-016.

Panel member	Bleed Date	Day	Panel Manufacturer's Data		*Vitros* result	
			HBeAg Abbott EIA	Anti-HBe Abbott EIA	HBeAg	Anti-HBe
1	17/07/96	1	0.26	0.56	0.12	0.17
2	24/07/96	8	0.2	0.66	0.10	0.18
3	26/07/96	10	0.22	0.67	0.10	0.20
4	02/08/96	17	0.18	0.71	0.10	0.22
5	08/08/96	23	0.21	0.70	0.11	0.21
6	10/08/96	25	0.22	0.53	0.13	0.16
7	11/09/96	57	**6.13**	0.53	**47.0**	0.05
8	14/09/96	60	**1.94**	0.71	**5.31**	0.16
9	28/09/96	74	0.29	**1.37**	0.11	**2.41**
10	03/10/96	79	0.25	**1.75**	0.11	**2.86**
11	05/10/96	81	0.25	**1.56**	0.10	**2.85**
12	12/10/96	88	0.19	**1.69**	0.10	**10.2**
13	31/10/96	107	0.21	**2.86**	0.10	**22.8**
14	02/11/96	109	0.30	**2.78**	0.10	**19.6**
15	07/11/96	114	0.25	**2.70**	0.10	**22.3**
16	09/11/96	116	0.30	**2.78**	0.10	**10.2**
17	14/11/96	121	0.30	**3.03**	0.09	**20.4**
18	16/11/96	123	0.22	**3.23**	0.10	**18.0**
19	21/11/96	128	0.15	**3.13**	0.10	**21.2**
20	20/12/96	157	0.27	**4.76**	0.10	**23.7**

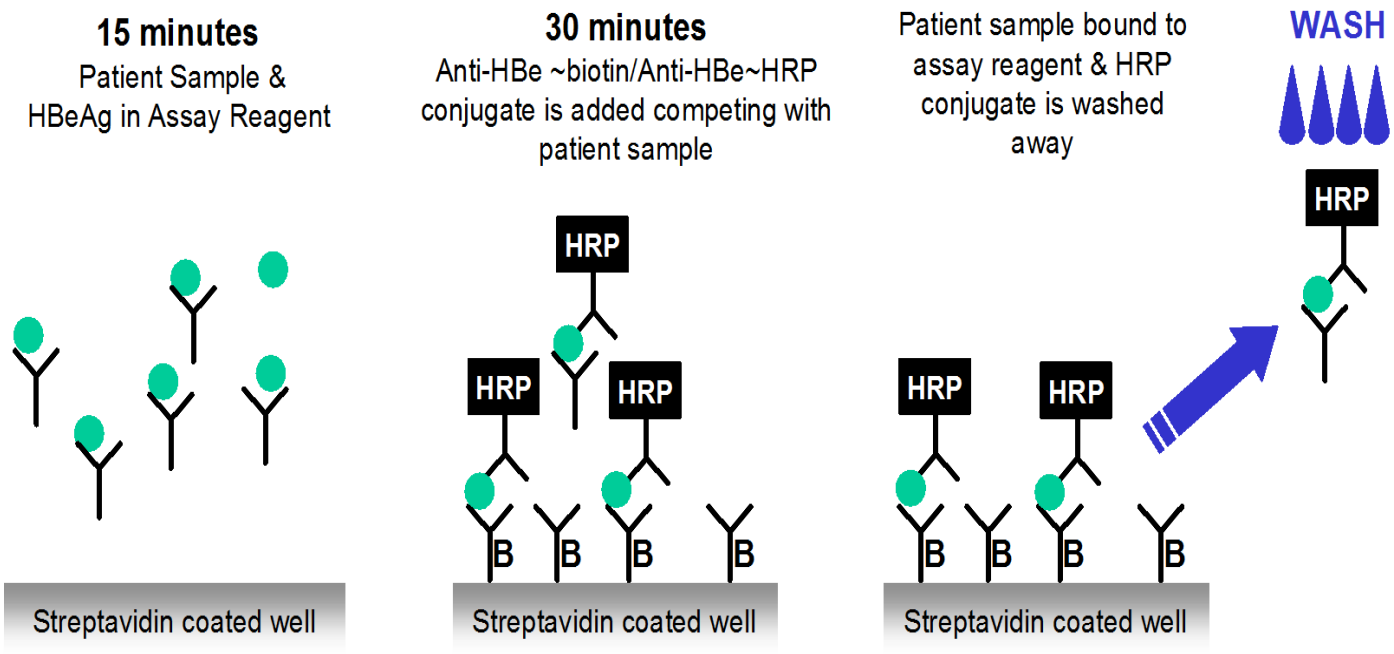
Results = signal/cut-off
= >1 positive

Vitros Anti-HBe assay

STEP 1:
15 minutes
Patient Sample &
HBeAg in Assay Reagent

STEP 2:
30 minutes
Anti-HBe ~biotin/Anti-HBe~HRP conjugate is added competing with patient sample

WASH STEP:
Patient sample bound to assay reagent & HRP conjugate is washed away



Response 1	wSQQText
30265	Negative
35526	Negative
20383	Negative
24133	Negative
25103	Negative
30819	Negative
3	Reactive
35554	Negative
3	Reactive
3	Reactive
34852	Negative
23789	Negative
26105	Negative

Patient sample (Anti-HBe)
Assay reagent (HBeAg)
Conjugate reagent (Anti-HBe~biotin) + *Conjugate reagent (Anti-HBe~HRP)*

Vitros Anti HBe Sensitivity

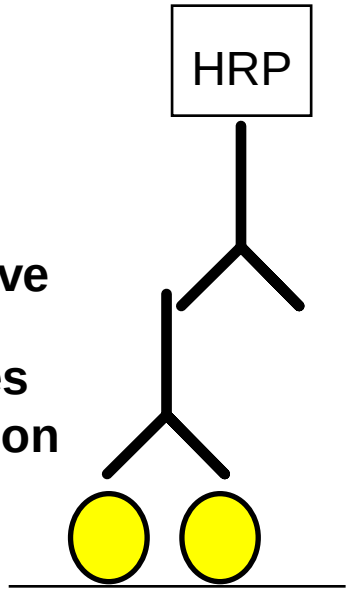
RP-009			Literature Data		*Vitros*	
			HBeAg	aHBe	HBeAg	aHBe
			Sorin Biomedica			
member	Bleed Date	Day	ETI-EBK	ETI-EBK		
1	18/07/95	1	0.5	0.52	0.12	0.17
2	21/07/95	3	0.95	0.53	0.24	0.16
3	29/07/95	11	1.19	0.52	1.41	0.12
4	31/07/95	13	1.17	0.5	2.36	0.14
5	16/08/95	29	21.53	0.39	1727	0.00
6	18/08/95	31	19.78	0.44	1988	0.00
7	23/08/95	36	21.15	0.37	1733	0.00
8	30/08/95	43	18.86	0.36	1264	0.00
9	12/09/95	56	25.67	0.37	1146	0.00
10	25/09/95	69	19.61	0.41	605	0.00
11	07/10/95	81	6.26	1.03	23.7	0.11
12	14/10/95	88	0.99	1.69	0.14	1.71
13	24/10/95	98	0.52	1.79	0.14	1.12
14	04/11/95	109	0.54	2	0.12	1.39
15	18/11/95	123	0.63	2.94	0.11	2.04
16	28/11/95	133	0.44	1.96	0.11	2.11
17	16/12/95	152	0.6	1.96	0.59	0.97
18	30/12/95	166	0.58	1.41	0.10	1.57
19	19/01/96	186	0.38	1.43	0.11	1.94
20	04/02/96	202	0.64	1.56	0.12	2.34

>1 positive

Anti HCV

Vitros Anti-HCV assay

- **20µl sample**
- **Two Step**
- **Antiglobulin**
- **Semi-Quantitative**
- **TTFR 55 minutes**
- **30 + 15 incubation**

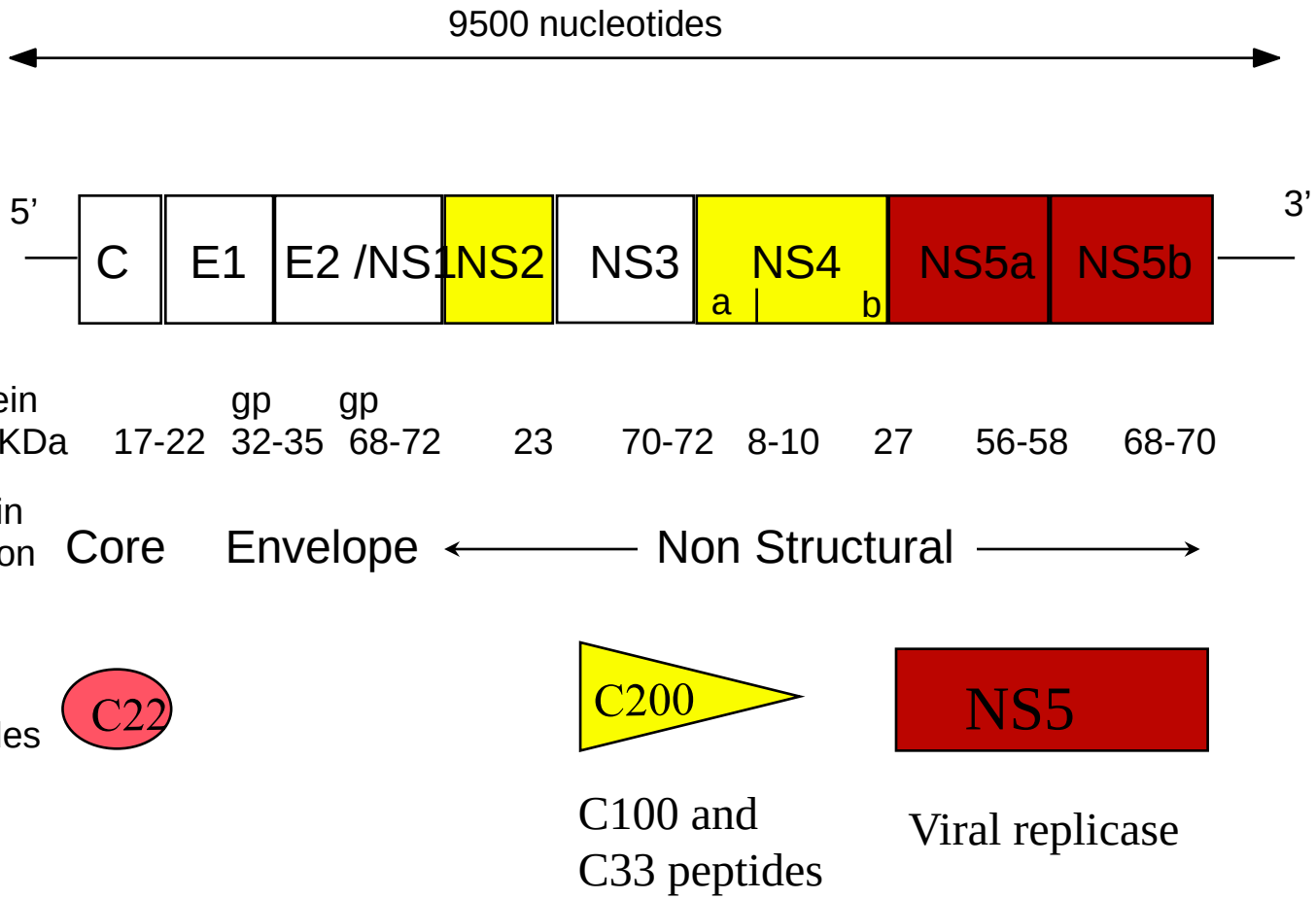


anti human IgG~HRP Conjugate (monoclonal)

Sample anti-HCV

Recombinant HCV antigens on Coated Well

Organisation of the HCV genome and encoded proteins

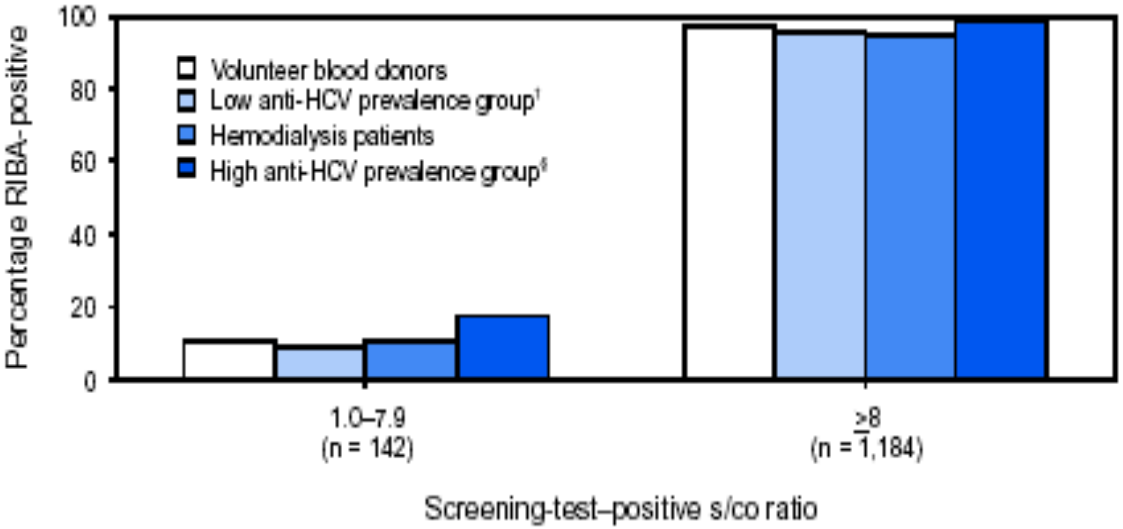


Vitros Anti-HCV Sensitivity - BCP Seroconversion Panel

Sample	Ortho vs 3.0	Ortho 3.0/20	AxSYM	AxSYM 3	Sanofi Access	*Vitros* ECi	RIBA (Ortho 3.0)					
							c100	c33c	c22	NS-5	SOD	Result
6211-37	0.00	0.02	0.37	NT	0.16	0.19	-	-	-	-	-	NEG
6211-38	0.85	0.80	0.37	NT	**1.04**	**2.02**	-	2+	-	-	-	IND
6211-39	**3.97**	**3.02**	0.40	NT	**2.28**	NT	1+	3+	-	-	-	POS
6211-40	**4.15**	**4.13**	0.49	NT	**3.17**	**11.9**	3+	4+	-	-	-	POS
6212 -1	0.00	0.01	0.61	NT	0.13	0.13	-	-	-	-	-	NEG
6212-2	0.15	0.43	**3.28**	NT	0.22	**1.66**	-	-	-	-	-	NEG
6212-3	0.30	0.65	**2.41**	NT	0.16	**1.90**	-	+/-	-	-	-	IND
6212-4	**1.49**	**2.26**	**3.96**	NT	0.54	**7.00**	-	1+	-	-	-	IND
6212-5	**1.87**	**3.24**	**3.93**	NT	**2.30**	**9.97**	-	1+	-	-	-	IND
6214-8	0.02	0.04	0.41	NT	0.16	0.23	+/-	+/-	-	-	-	NEG
6214-9	0.90	0.68	0.46	NT	0.55	**2.49**	+/-	2+	-	-	-	IND
6214-10	**2.64**	**1.83**	0.53	NT	**1.64**	**6.81**	+/-	3+	-	-	-	IND
6214-11	**4.13**	**4.09**	**1.25**	NT	**>6.68**	**20.6**	2+	4+	-	-	-	POS

aHCV specificity

FIGURE 2. Proportion of antibody to hepatitis C virus (anti-HCV) enhanced chemiluminescence immunoassay* screening-test–positive results that tested recombinant immunoblot assay (RIBA®) 3.0-positive by signal-to-cut–off (s/co) ratios and group tested



*VITROS® Anti-HCV assay.
†College students, general population, and health-care workers.
§Hospital-based patients.

US/EU Clinical Trial Data

Sensitivity: 100%

Seroconversion Sensitivity:

Superior early seroconversion sensitivity in 14 of 20 panels tested.

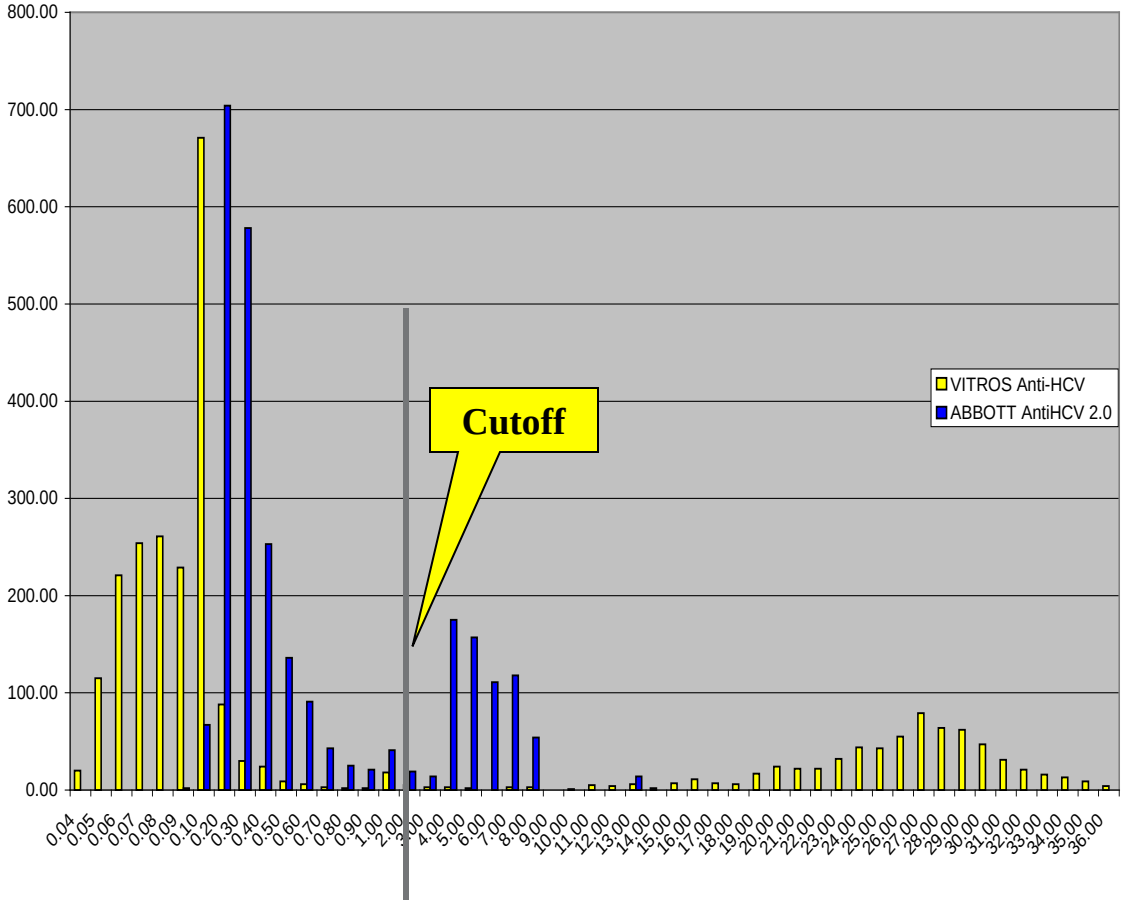
Excellent genotype detection:

26/26 samples reactive (1a, 1b, 2a/c, 3a/b, 4c/d, 4h, 5a and 6)

Specificity

Donor* 99.76%
(5361/5374)

Clinical** 98.22%
(1930/1965)



* data from ex-US studies
** data from US studies

Vitros Anti-HCV Sensitivity - BBI Seroconversion Panel

Sample	Ortho vs 3.0	Ortho 3.0/20	AxSYM	AxSYM 3	Sanofi Access	*Vitros* ECi	RIBA (Ortho 3.0)					
							c100	c33c	c22	NS-5	SOD	Result
905-4	0.50	0.45	0.53	0.85	0.35	0.23	-	1+	-	-	-	IND
905-5	0.90	0.99	NT	**1.16**	0.53	0.36	-	1+	-	-	-	IND
905-6	**1.60**	**1.65**	0.50	**1.81**	**1.05**	**1.00**	-	1+	+/-	-	-	IND
905-7	**3.80**	**2.86**	0.42	**4.31**	**2.33**	**4.74**	-	2	1+	-	-	POS
905-8	**>3.8**	**4.17**	PS	**21.07**	**4.54**	**NT**	-	+ 4+	4+	-	-	POS
906-1	**5.40**	**2.82**	0.76	NT	**2.53**	**4.90**	+/-	4+	-	-		IND
906-2	**6.40**	**3.23**	0.66	NT	**2.48**	**7.26**	+/-	4+	-	-		IND
906-3	**>7.8**	**4.17**	0.57	NT	**5.26**	**10.1**	2+	4+	-	-	-	POS
907-3	0.00	0.03	NT	0.13	0.17	0.16	-	-	-	-	-	NEG
907-4	0.10	0.07	NT	0.26	0.27	**1.68**	-	-	1+	-	-	IND
907-5	0.40	0.46	NT	**1.49**	**1.19**	**7.82**	-	+/-	4+	-	-	IND
907-6	**1.00**	**1.27**	NT	**3.88**	**2.20**	**11.7**	-	1+	4+	-	-	POS

Vitros Anti-HCV Sensitivity - NABI Seroconversion Panel

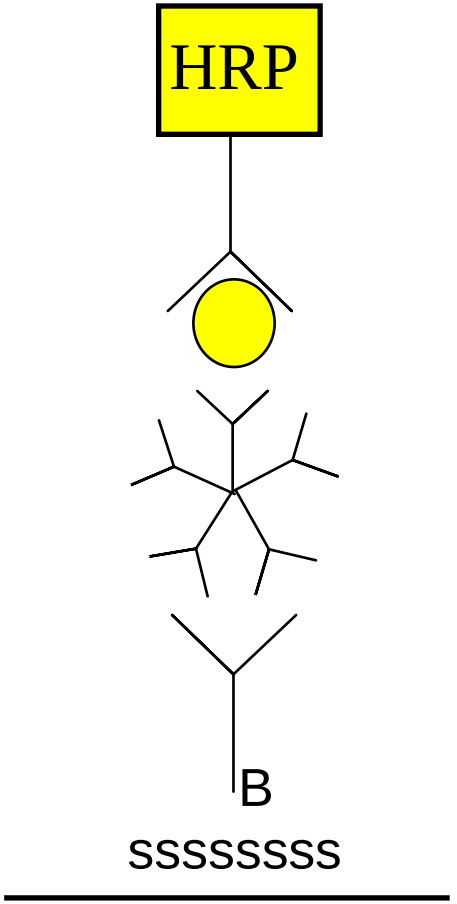
Sample	Ortho vs 3.0	Ortho 3.0/20	AxSYM	AxSYM 3	Sanofi Access	*Vitros* ECi	RIBA (Ortho 3.0)					
							c100	c33c	c22	NS-5	SOD	Result
SC040-2	0.06	NT	0.43	NT	0.14	0.44	-	-	-	-	-	NEG
SC040-3	**1.22**	NT	0.40	NT	0.85	**5.58**	+/-	2+	-	-	-	IND
SC040-4	**1.53**	NT	0.40	NT	**1.56**	**11.6**	+/-	2+	-	-	-	IND
SC010-1	0.01	0.02	AS	AS	AS	0.04	-	-	-	-	-	NEG
SC010-2	0.17	0.15	AS	AS	AS	**2.62**	-	-	2+	-	-	IND
SC010-3	**1.30**	**1.53**	AS	AS	AS	**15.5**	-	+/-	4+	-	-	IND
SC030-1	0.01	0.02	AS	AS	AS	0.03	-	-	-	-	-	NEG
SC030-2	0.02	0.20	AS	AS	AS	**2.68**	3+	+/-	+/-	-	-	IND
SC030-3	**1.84**	**1.87**	AS	AS	AS	**10.7**	4+	1+	2+	-	-	POS
SC030-4	**>4.9**	**4.04**	AS	AS	AS	**34.7**	4+	4+	4+	3+	-	POS

Immunocapture et IgM

Assay design

Result classification:

<0.8	**Negative**
>1.2	**Positive**
≥0.8 -1.19	**Borderline**



Recombinant HBcAg~HRP/ antibody conjugate complex

Sample anti-HBc specific IgM

Biotinylated Anti-human IgM

Streptavidin coated well

HBV Seroconversion and aHBc IgM results

Sample No	Day	Vitros	AxSYM	Corzyme-M
1	1	0.02	0.07	0.08
2	8	0.01	0.08	0.10
3	10	0.02	0.11	0.13
4	17	0.02	0.10	0.12
5	23	0.02	0.08	0.11
6	25	0.01	0.08	0.08
7	57	0.36	0.33	0.52
8	60	1.86	1.72	3.48
9	74	3.57	1.98	4.36
10	79	3.39	2.02	4.10
11	81	3.07	1.86	3.96
12	88	2.69	1.79	3.50
13	107	1.83	1.56	2.72
14	109	1.57	1.41	2.54
15	114	1.40	1.17	2.51
16	116	1.23	1.09	1.97
17	121	1.14	0.97	2.03
18	123	1.03	0.88	1.67
19	128	0.96	0.86	1.80
20	157	0.95	0.91	1.70

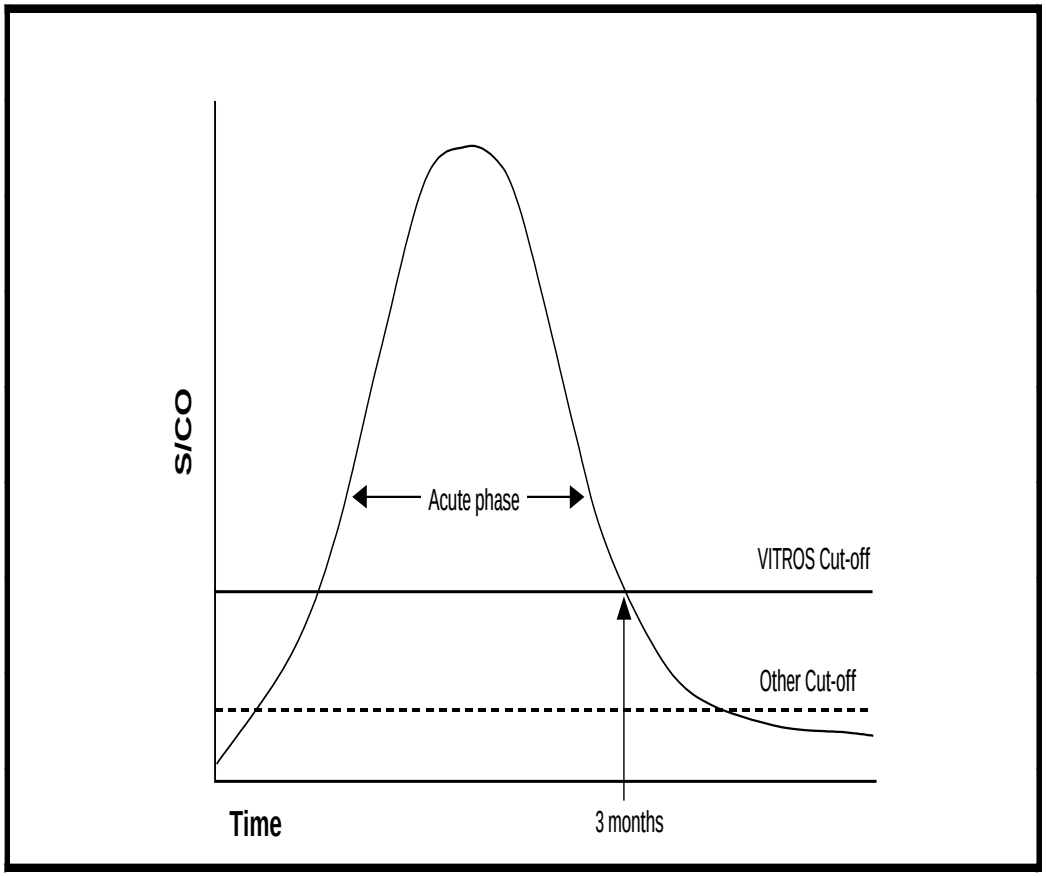
The Vitros assay has a level of sensitivity which is appropriate for its intended use.

This sensitivity is similar to Abbott AxSYM.

Abbott Corzyme is more sensitive and may be reactive in chronic cases.

Discrepancies with other assays can only be resolved using an accurate clinical history.

Sensitivity summary



Toxo IgM assay design

10µl sample + 190 µl **HSDB**

20 µL of the **diluted sample**

140 µL Assay Reagent (Biotinylated anti-human IgM)

16 min incubation

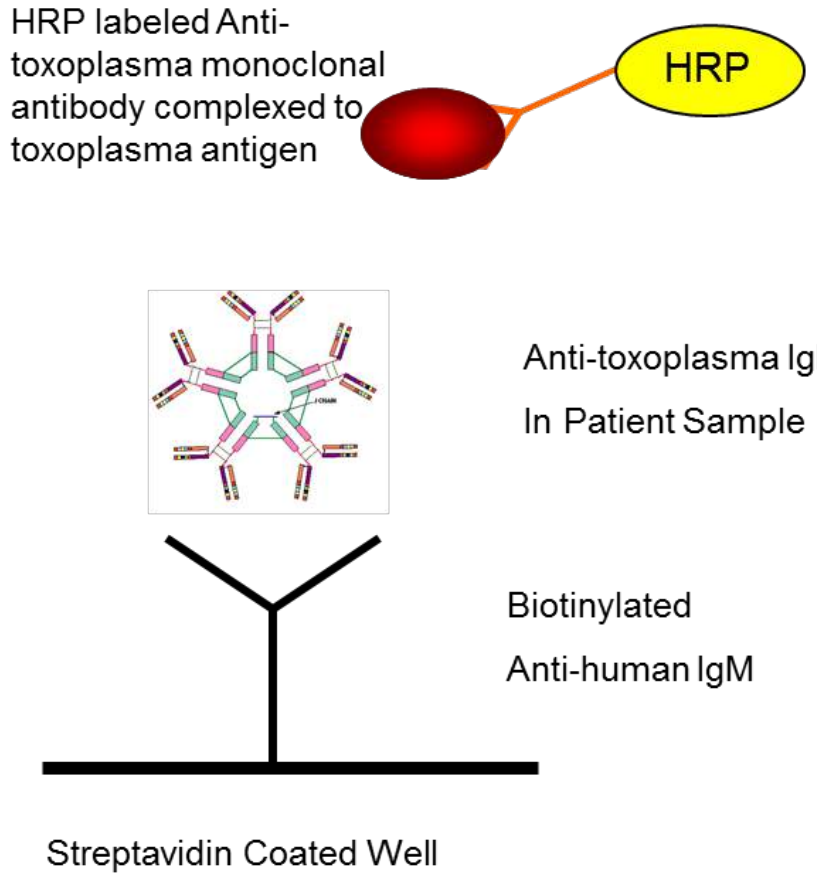
Wash (protocol 2)

160 µL Conjugate Reagent (HRP labeled anti-toxoplasma complexed to toxoplasma antigen)

16 min incubation

Well is washed (protocol 2), Signal Reagent is added and well is read.

2 Step Immunometric Class Capture Assay



CMV IgM assay design

10µl sample + 190 µl **HSDB**

20 µL of the **diluted sample**

140 µL Assay Reagent (Biotinylated anti-human IgM)

8 min incubation

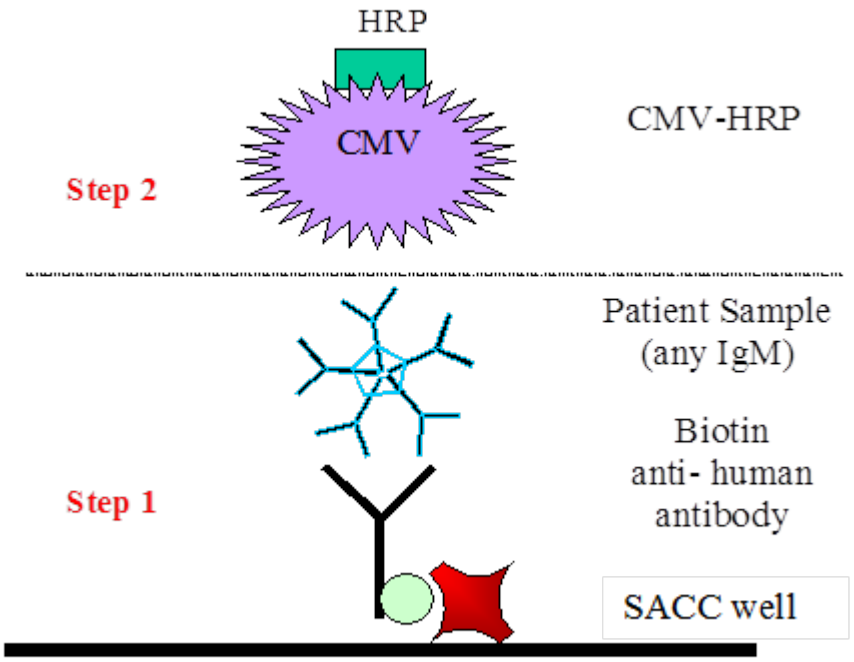
Wash (protocol 2)

160 µL Conjugate Reagent (HRP labeled inactive CMV antigen)

48 min incubation

Well is washed (protocol 2), Signal Reagent is added and well is read.

2 Step Immunometric Class Capture Assay

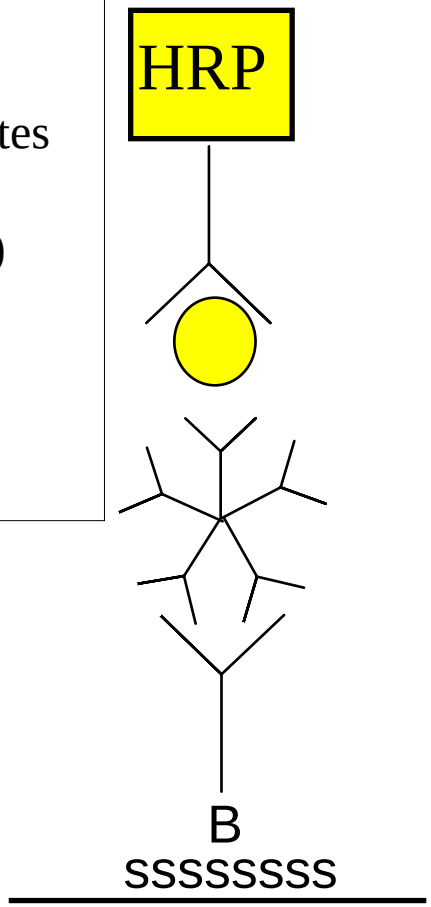


Assay design Anti HAV IgM

Sample volume: 10 µL
Diluent pack **Diluent B***
Calibration interval: **14 days**
Incubation time: 15 + 15 minutes
Time to 1st result: 40 minutes
Specificity: 508 Donor (100%)
60 Clinical (100%)
Sensitivity: 99.6%

*sample pre-dilution 1:20

Result classification:
<0.8 Negative
≥ 1.2 Positive
≥ 0.8 to <1.2 Borderline



Inactivated HAV antigen~HRP/ antibody conjugate complex

Sample anti-HAV specific IgM

Biotinylated Anti-human IgM

Streptavidin coated well

Comparitive Seroconversion sensitivity

Seroconversion Panel – 01010

Panel Member	Bleed Date	Day	*Vitros* Anti-HAV IgM assay result	Abbott HAVAB-M® assay result	Roche Elecsys Anti-HAV IgM assay
1	4/4/96	1	0.02	0.18	0.37
2	9/4/96	6	0.02	0.20	0.41
3	12/4/96	9	**3.96**	1.06	**4.57**
4	16/4/96	13	**6.98**	**6.58**	**36.11**
5	8/5/96	35	**7.04**	**7.39**	**30.37**
6	24/5/96	51	**5.90**	**4.69**	**12.07**
7	9/6/96	67	**4.05**	**2.07**	**4.47**
8	27/6/96	85	**2.83**	**1.89**	**2.79**
9	14/7/96	102	**1.90**	0.64	**1.76**
10	2/8/96	121	**1.62**	0.86	**1.49**
11	15/8/96	134	1.14	0.81	**1.30**
12	29/8/96	148	0.88	0.83	1.15
13	12/9/96	162	0.77	0.77	1.06
14	30/9/96	180	0.63	0.64	0.99
15	10/10/96	190	0.55	0.65	0.91

Tests combo
Antigen/anticorps
exemple HIV combo

The Evolution of HIV Immunoassays (IA)

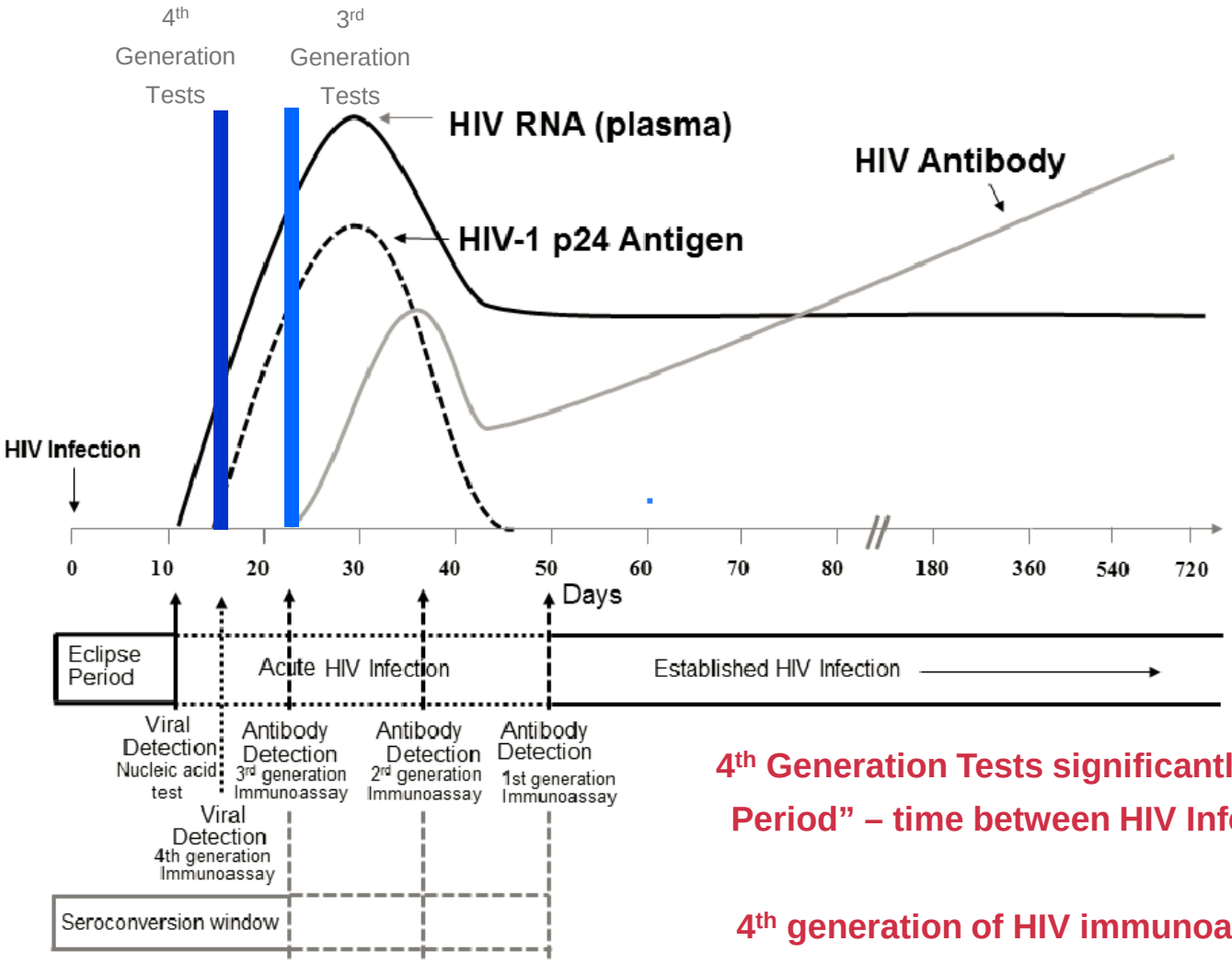
Assays	Coating Material	Detection
1st generation	Viral culture cell lysates	HIV-1 IgG antibody
2nd generation	Viral culture cell lysates or Synthetic/Recombinant antigen	HIV-1/2 IgG antibody
3rd generation	Synthetic/Recombinant antigen	HIV-1/2 IgM/IgG antibody
4th generation	Synthetic/Recombinant antigen and anti-p24 antibody	HIV-1/2 IgM/IgG antibody and p24 antigen

The evolution of the HIV IA significantly increased assay sensitivity and reduced the Window Period, which is the time between HIV infection and detection.

The 4th generation HIV IA can detect HIV infection at early acute phase

CDC and APHL. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations. June 27, 2014.

Laboratory Stages of HIV infection



4th Generation Tests significantly reduce the "Window Period" – time between HIV Infection and Detection*

4th generation of HIV immunoassays can detect HIV infection ~ 2 weeks post exposure*

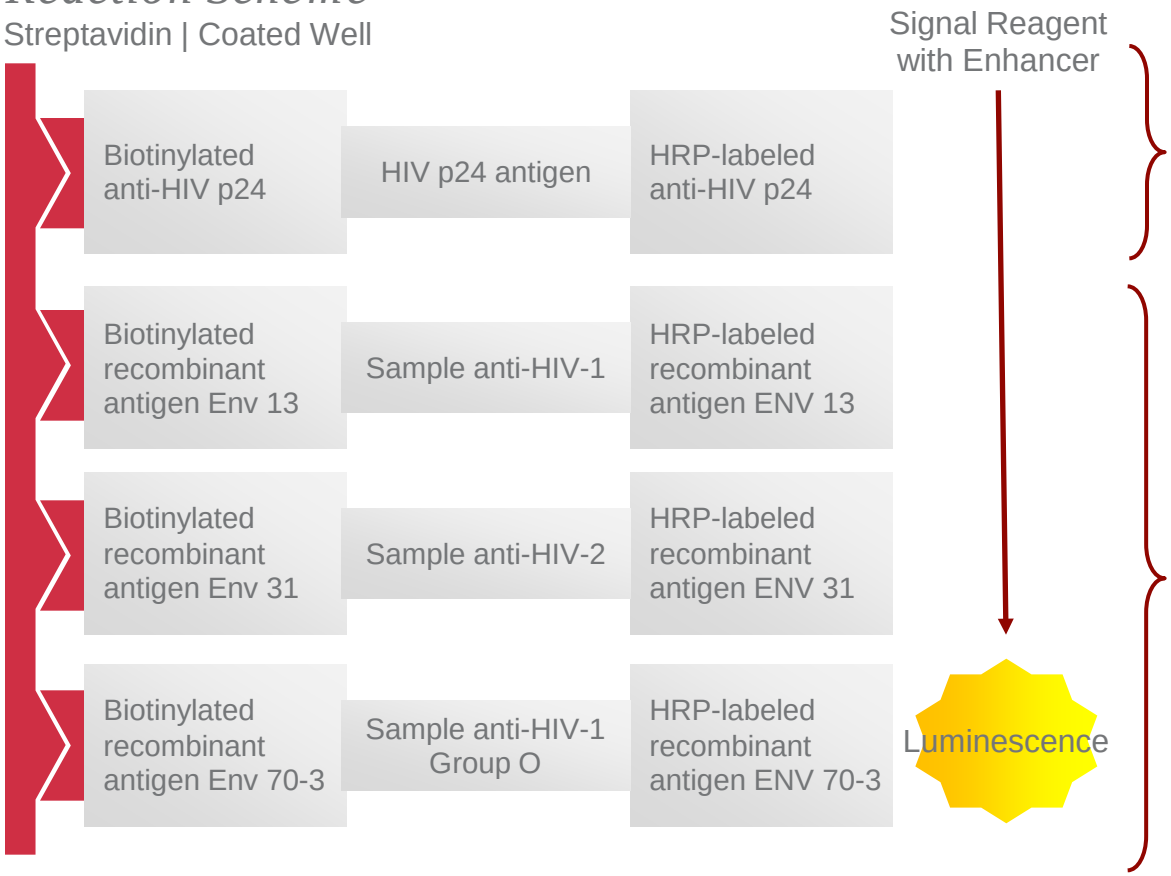
**CDC and APHL. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations. June 27, 2014.*

VITROS® HIV Combo Assay Structure and Reaction Scheme*

Acquired Immunodeficiency Syndrome (AIDS) is caused by at least two types of Human Immunodeficiency Viruses designated HIV-1 and HIV-2.

Reaction Scheme

Streptavidin | Coated Well



Simultaneous Antigen and Antibody Detection

Antigen Detection

The test also uses monoclonal antibodies to detect HIV-1 p24 antigen, present in blood before the onset of antibody response, thus enabling earlier diagnosis of HIV-1 infection.

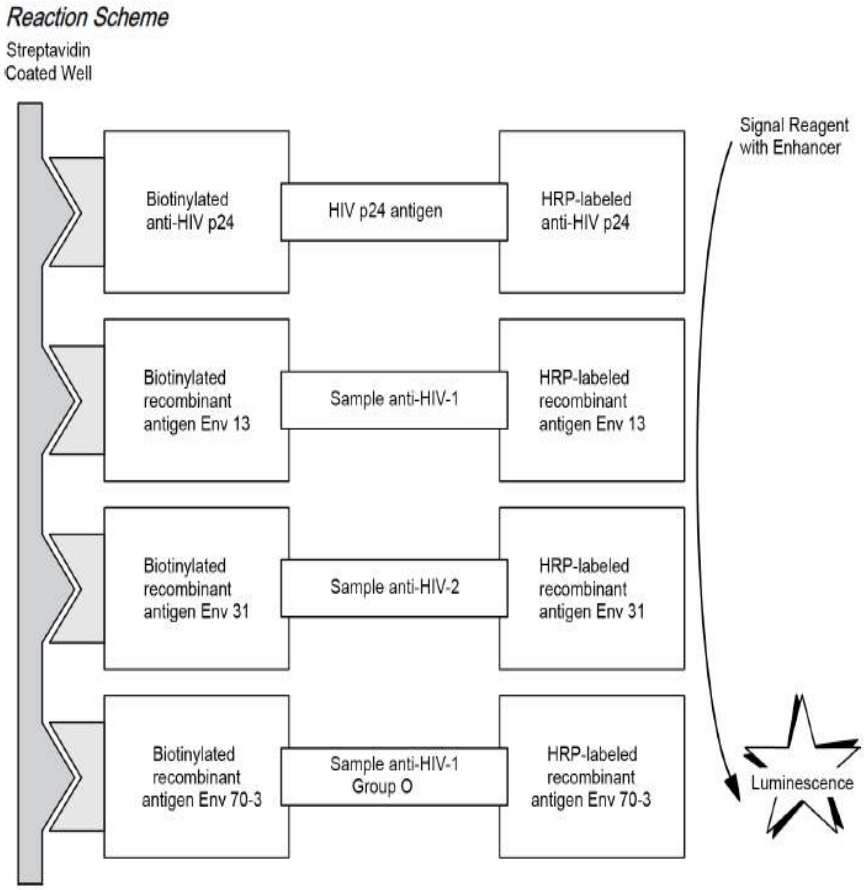
Antibody Detection

The test uses 3 recombinant antigens derived from three HIV envelopes (two from HIV-1 including group O and one from HIV-2).

These antigens detect IgM and IgG antibodies to HIV-1 and HIV-2 in the same test.

*VITROS® Immunodiagnostic Products HIV Combo Assay IFU

VITROS® HIV Combo
Assay Structure and Reaction Scheme*



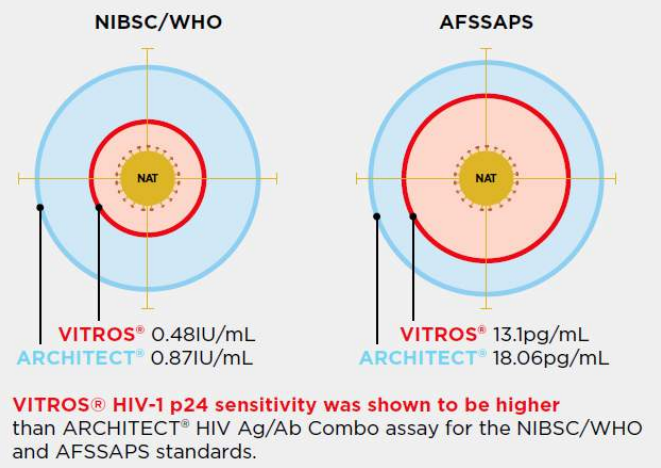
- Immunometric Technique
- Two-stage reaction.
- Incubation Time: 37 mins
- Time to First Result: 48 mins
- Reaction Sample Volume: 80 µL

**VITROS® Immunodiagnostic Products HIV Combo Assay IFU*

VITROS® HIV Combo – Class-Leading Analytical Sensitivity – p24 Detection*

Analytical Sensitivity
NIBSC/WHO : 0.48IU/mL
AFSSAPS : 13.1pg/mL

Provides assurance in detecting infection



VITROS® HIV-1 p24 sensitivity was shown to be higher than ARCHITECT® HIV Ag/Ab Combo assay for the NIBSC/WHO and AFSSAPS standards.

Detection of HIV-1 viral nucleic acid with Nucleic Acid Test (NAT) remains the most sensitive method in identifying acute HIV-1 infection but its use is not widespread due to associated cost, time and labor.

VITROS® HIV Combo detected p24 at lower concentrations than a leading commercially available test

*VITROS® Immunodiagnostic Products HIV Combo Assay IFU

VITROS® HIV Combo
Clinical Performance – Specificity*

Samples from **5077** presumed healthy blood donors, and **608** clinical specimens were tested at two external sites in the
VITROS HIV Combo test and another commercially available CE marked 4th generation Ag/Ab Combo Test.

The specificity of the VITROS HIV Combo test for the donor population was calculated as **99.84% (5069/5077) exact 95% CI (99.69-99.93%). The specificity of the VITROS HIV Combo test for the clinical population was calculated as 100.00%** (607/607) exact 95% CI (99.39-100.00%).

Samples	Number of test samples	Initially Reactive	Repeatedly Reactive	Confirmed Reactive
Donor	5077	16	8	**0**
Clinical	**608**	**1**	**1**	**1#**

Sample confirmed as Reactive in a 3rd generation antibody immunoassay, a line immunoassay and a nucleic acid test (NAT). This sample was excluded from the calculation of specificity.

**VITROS® Immunodiagnostic Products HIV Combo Assay IFU*

VITROS® HIV Combo
Clinical Performance – Seroconversion Sensitivity*

Thirty four commercially available seroconversion panels tested on **VITROS HIV Combo** and **a commercially available 4th generation Ag/Ab combo** test.

The table presents the number of reactive panel members, the days from first bleed to first reactive result and the difference in days to first reactive between the two tests.

The VITROS HIV Combo Test and the commercially available 4th generation Ag/Ab Combo Test were in agreement for **28 of the 34 panels**. **The VITROS HIV Combo Test became reactive one bleed earlier for five of the thirty four panels. The commercially available 4th generation Ag/Ab Combo Test became reactive one bleed earlier for one panel.**

Days to Evidence of HIV Infection

	Number of Reactive Panel Members		Days to First Reactive Result		
Panel ID	VITROS HIV Combo Test	Commercially Available 4th Generation HIV Ag/Ab Test	VITROS HIV Combo Test	Commercially Available 4th Generation HIV Ag/Ab Test	Difference in Days to First Reactive Result*
PBR934	3	3	0	0	0
PBR950	3	2	18	21	3
PBR954	2	2	17	17	0
PBR966	3	3	44	44	0
6243	4	4	24	24	0
6244	2	2	27	27	0
6247	4	4	21	21	0
6248	2	2	18	18	0
9021	4	4	46	46	0
9079	17	17	40	40	0
HIV9012	4	3	14	16	2
HIV9014	6	6	0	0	0
HIV9077	16	16	42	42	0
HIV9020	3	3	89	89	0
HIV9018	4	3	31	34	3
HIV9015	2	2	30	30	0
PBR955	4	4	3	3	0
PBR930	4	4	0	0	0
PBR951	4	4	8	8	0
PBR963	2	2	17	17	0
HIV12007	6	6	117	117	0
HIV12008	6	5	23	28	5
HIV9013	1	1	25	25	0
HIV9028	2	2	53	53	0
HIV9032	8	7	22	24	2
HIV9075	3	3	22	22	0
HIV9089	3	3	16	16	0
PRB943	5	5	7	7	0
PRB956	2	2	47	47	0
PRB957	2	3	23	16	–7
PRB960	2	2	28	28	0
PRB961	2	2	21	21	0
PRB962	2	2	14	14	0
PRB964	1	1	22	22	0
Total	138	134	929	937	8

* Days to first reactive test result on the commercially available test minus the days to first reactive test result for the VITROS HIV Combo test

Did You Know?

Seroconversion panels are a group of serial bleeds from plasma donors during the early stages of infection. They are intended for use by manufacturers and clinical laboratories to evaluate assay sensitivity.

**VITROS® Immunodiagnostic Products HIV Combo Assay IFU*

VITROS® HIV Combo – Excellent Precision*

Performed using two reagent lots on two different VITROS® 3600 systems.

Data on VITROS® 5600 and ECi/ECiQ is found in IFU

Panel Member	Mean S/C	Within-run* CV (%)	Within-calibration** CV (%)	Within-lab*** CV (%)
Anti-HIV-1	0.56	4.4	9.3	9.3
Anti-HIV-1	0.98	3.2	7.8	7.6
Anti-HIV-1	2.17	2.7	5.7	5.4
Anti -HIV-1 Reactive Control	1.90	3.8	6.1	6.0
Anti-HIV-2	0.72	5.1	10.1	10.0
Anti-HIV-2	1.04	3.7	7.3	7.0
Anti-HIV-2	2.44	3.1	5.4	5.1
Anti -HIV-2 Reactive Control	4.20	3.0	4.6	4.5
Anti-HIV-1 Group O	0.86	5.2	8.7	8.7
Anti-HIV-1 Group O	1.10	4.2	7.3	7.3
Anti-HIV-1 Group O	2.38	4.0	5.6	5.6
Anti -HIV-1 Group O Reactive Control	3.30	3.3	5.3	5.0
HIV p24 Ag	0.78	2.3	7.4	7.5
HIV p24 Ag	1.40	2.0	5.3	5.4
HIV p24 Ag	3.33	1.5	3.3	3.4
HIV p24 Ag Reactive Control	1.92	1.7	4.7	4.7

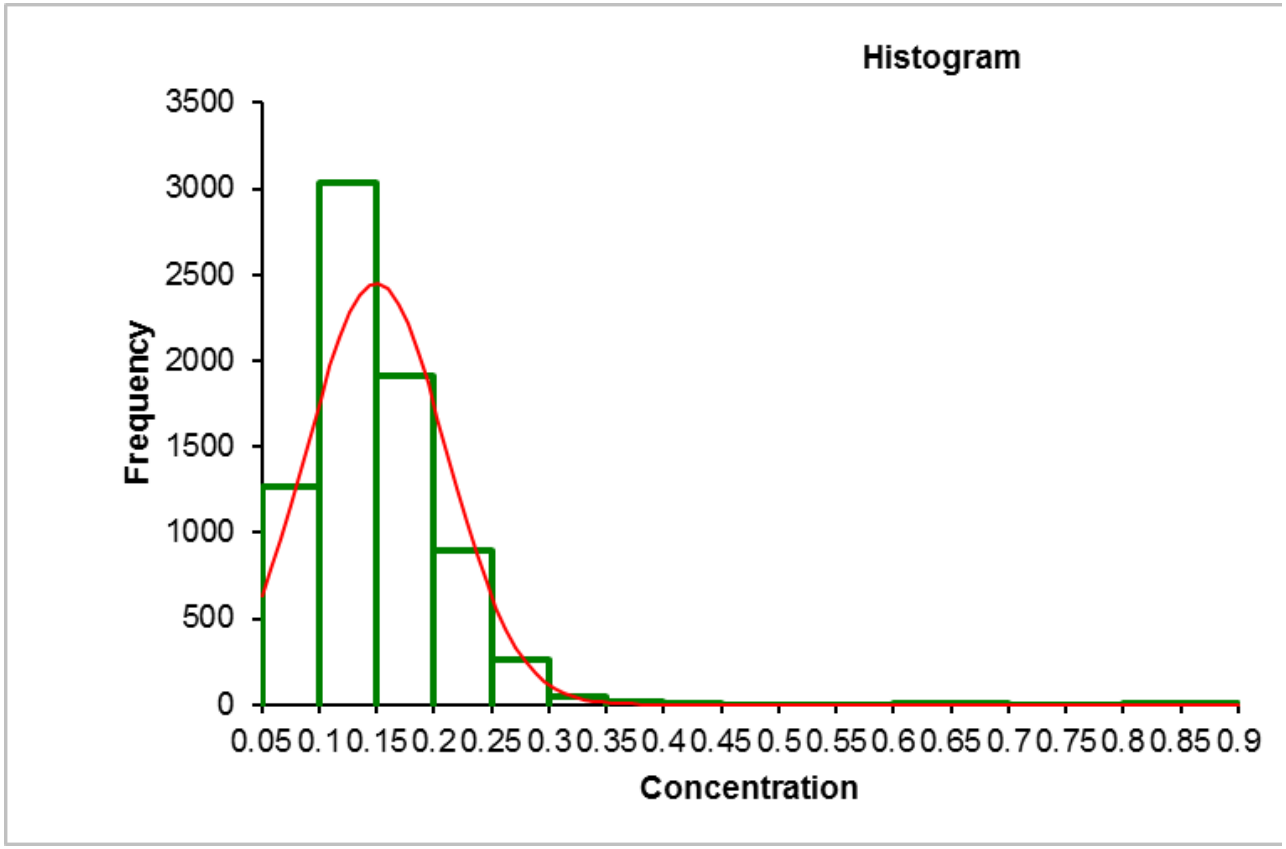
Reliable results due to excellent precision.

*VITROS® Immunodiagnostic Products HIV Combo Assay IFU

What have we learnt from econn customer data?

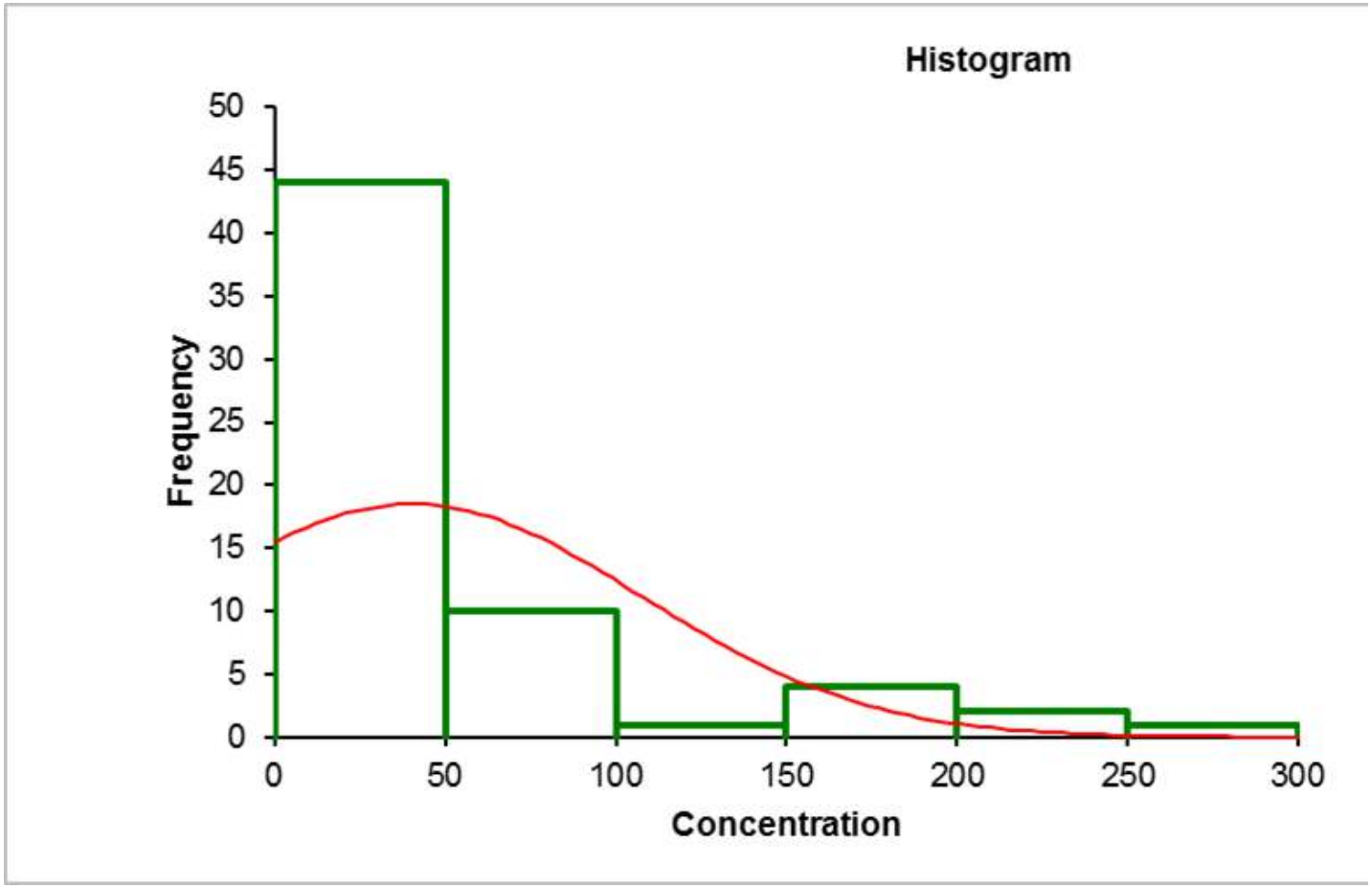
Negative sample distribution: N= 7464; mean 0,15 s/co

Very few borderline results (0.03%, 3 out of 7529)



What have we learnt from econn customer data?

Positive sample distribution: N= 62; mean ….?



Lot to lot variation

		ORTHO Reagent lots	20	31	41	50	60	70	80	90	100	110	115	120	125	130	140	160	170	181	190
Product	Type	Cat #																			
		Bio-Rad lot number	107650									107630						107640			
Bio-Rad VIROCLEAR®	Negative Control	00106 or 00112	0.14	0.12	0.14	0.14	0.11	0.15	0.19	0.14	0.20	0.15	0.10	0.10	0.17	0.11	0.12	0.14	0.12	0.08	0.12
Bio-Rad VIROTROL® I	Anti-HIV-1 positive control	Bio-Rad lot number	119050							119060											
		00100E or 00101E	2.14	2.26	2.29	2.14	2.13	2.47	2.16	1.90	1.79	1.67	1.77	1.78	1.69	1.66	1.95	1.56	1.67	1.87	1.75
Bio-Rad VIROTROL® HIV-2	anti-HIV-2 positive control	Bio-Rad lot number	114830						114840			114860									
		00105C	5.16	5.58	5.23	4.46	4.59	4.78	4.52	4.95	4.82	3.92	1.98	1.89	2.03	1.77	1.93	1.72	1.87	1.94	2.3
Bio-Rad VIROTROL® HIV-1 Ag	p24 antigen positive control	Bio-Rad lot number	114090					114130													
		00108A	1.65	1.59	1.51	1.54	1.59	3.91	3.48	3.45	3.55	3.29	3.43	3.35	3.47	3.39	3.42	3.34	3.38	3.50	3.18
Bio-Rad VIROTROL® HIV-1 gO	anti-HIV-1 gO positive control	Bio-Rad lot number	R0416010														39000	39010	38010		
		00113X or 113	3.49	3.54	3.14	3.16	2.80	3.48	2.92	3.15	3.25	2.86	3.16	2.98	3.21	2.68	3.14	3.09	3.29	3.65	3.75

Contrôles

Différents contrôles: Immuno-analyse

Noms	Molécules
VITROS® Immunodiagnostic Products Free Thyroid Controls	TSH Free T4 Free T3
VITROS® Immunodiagnostic Products Total Thyroid Controls	TSH Total T3 Total T4 T3 Uptake
VITROS® Immunodiagnostic Products RE Controls	LH FSH E2 AFP Prolactin Progesterone Total ßhCG II
VITROS® Immunodiagnostic Products Anemia Controls	Vit B12 Ferritin
VITROS® Immunodiagnostic Products Metabolism Controls	Cortisol
VITROS® Immunodiagnostic Products NTx Controls	NTx
VITROS® Immunodiagnostic Products Intact PTH Controls	iPTH
VITROS® Immunodiagnostic Products Testo Controls	Testo
VITROS® Immunodiagnostic Products Anti-HCV controls	aHCV neg aHCV pos
VITROS® Immunodiagnostic Products Anti-HIV Controls	aHIVneg et aHIV 1 pos et aHIV 2 pos
VITROS® Immunodiagnostic Products Anti-HAV IgM Controls	aHAVM pos et aHAVM neg
VITROS® Immunodiagnostic Products Anti-HBs Controls	aHBs 3 niveaux et pour neg voir Bio-Rad Viroclear
VITROS® Immunodiagnostic Products Anti-HBc Total Controls	aHBc neg et aHBc pos
VITROS® Immunodiagnostic Products Anti-HBc IgM Controls	aHBcM neg et aHBcM pos
VITROS® Immunodiagnostic Products HBs Ag ES Controls	HBsAg neg et HBsAg pos
VITROS® Immunodiagnostic Products HBe Controls	HBe Ag: 2 neg, 2 pos et Anti-HBe: 2 neg, 2 pos
VITROS® Immunodiagnostic Products Syphilis TPA Controls	Syphilis TPA
Cardiac Marker Control - CLINIQA	CK-MB Myoglobin Troponin I ES NTproBNP
Fujirebio Diagnostics Tumor Marker Controls	AFP CA 125 II CA 15-3 CA 19-9 CEA PSA PSA libre Ferritin

Moyenne de Réference et Ecart Type

- Lors de la création du fichier CQ dans le logiciel de l'analyseur, utiliser la moyenne et l'écart type fourni par le feuillet CQ

Vitros Anti-HIV 1+2 Controls - Baseline Statistics		
Control	Mean Result	SD
1 anti-HIV 1+2 negative	0.10	0.10
2 anti-HIV 1 positive	6.98	1.626
3 anti-HIV 2 positive	5.18	1.207

Statistiques d’enregistrement

- Moyenne & écart-type réels des résultats de CQ , calculés par le logiciel
- Possibilité de filtrer les résultats de CQ par date
- Vérification de l’exactitude:
- Toutes les valeurs de CQ doivent se situer dans l’intervalle suivant: 2 écarts-types de part et d’autre de la moyenne du feuillet CQ
- Comment vérifier la précision?
- L’ écart-type calculé doit être < ou = à l’ écart-type du feuillet CQ

- **Note** : l’ écart-type intra-laboratoire du feuillet technique permet une meilleure évaluation de la précision attendue d’un lot de réactif

Feuillet Contrôle

Calcule L'intervalle de Moyennes (R.O.M): Moyenne +/- 2 ET
2.2 – 6.1

Feuillet Réactif

Utilisé comme ET de référence après 20 jours

Valeurs de référence

Chaque moyenne mentionnée a été obtenue à partir d'un minimum de 10 dosages. L'écart type correspond aux résultats attendus pour un seul dosage de chaque contrôle et obtenus dans divers laboratoires à partir de lots de réactif différents. Les valeurs mentionnées sont propres à chaque lot.

Contrôles Anti-VIH 1+2 *Vitros* - Valeurs de référence

Contrôle	Moyenne	ET
1 anti-VIH 1+2 négatif	0,10	0,10
2 anti-VIH 1 positif	6,39	1,489
3 anti-VIH 2 positif	4,49	1,046

Précision

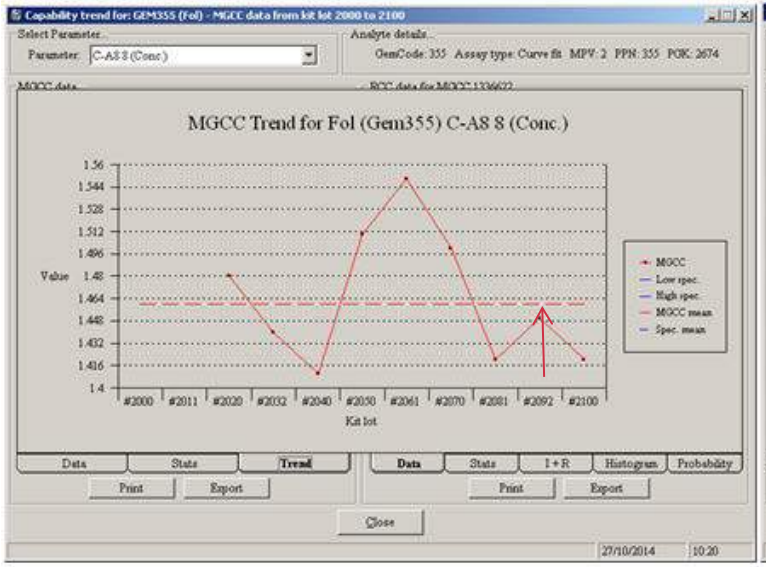
La précision a été évaluée à l'aide d'une méthode basée sur le protocole EP5-T2[5] du National Committee for Clinical Laboratory Standards. Deux exemplaires de chacun des 4 échantillons du groupe ont été dosés une fois par jour pendant au moins 20 jours, et ce, en utilisant 3 lots de réactif sur différents systèmes. Les résultats obtenus sont représentatifs des performances du produit.

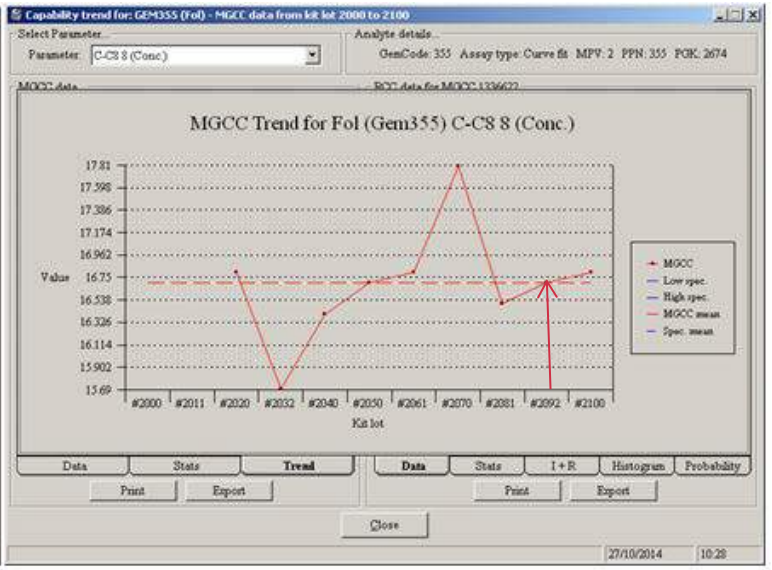
Tableau 1: Précision

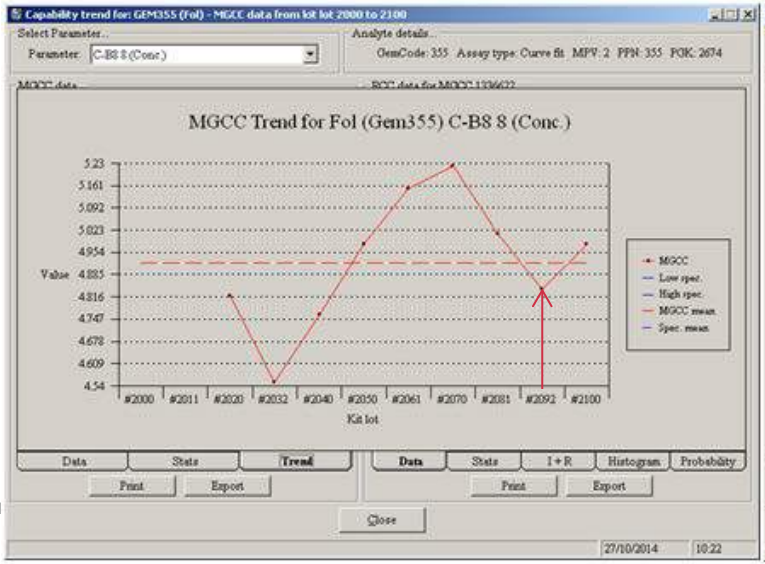
Résultat représentatif	Intra dosage écart type*	Intra dosage CV(%)*	Intra étalonnage écart type*	Intra étalonnage CV(%)*	Intra laboratoire écart type*	Intra laboratoire CV(%)*
0,15	0,00694	4,9	0,0118	8,2	0,00984	6,6
4,26	0,0789	1,9	0,157	3,8	0,213	4,5
4,60	0,0617	1,3	0,129	2,9	0,191	4,1
1,01	0,0154	1,6	0,0392	4,4	0,0467	4,7

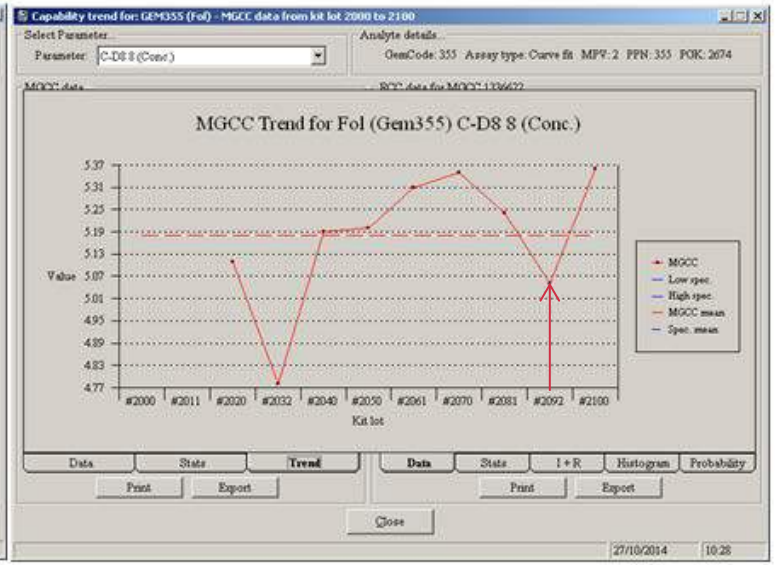
*Valeur moyenne quadratique (RMS)

Suivi interne de contrôles(ex: folates)

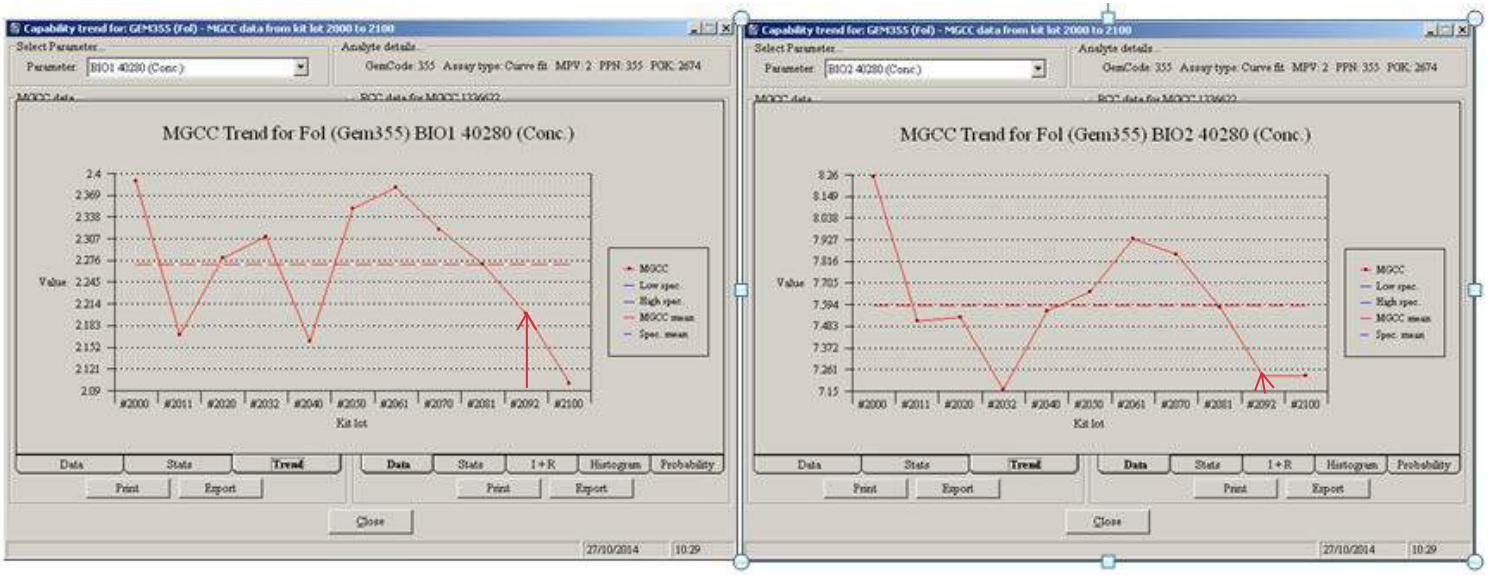


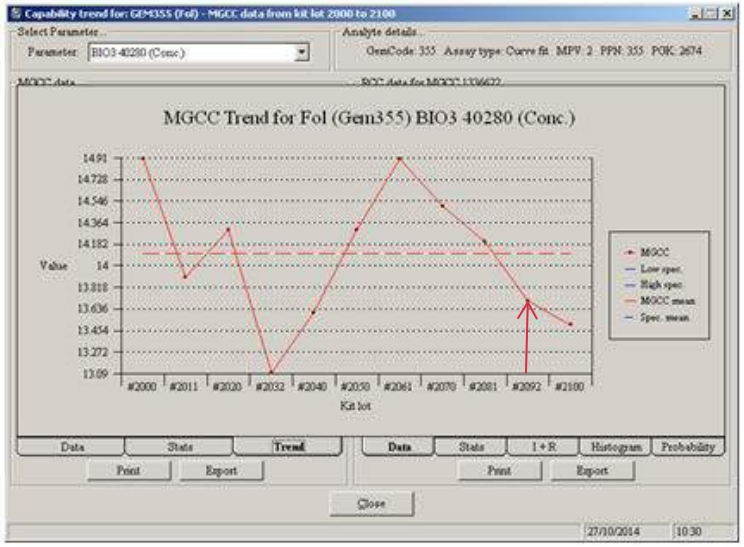






Suivi interne de contrôles(ex: folates)





Particularités en Virologie/Sérologie

- Tous nos seuils sont normalisés à **1,00**
- Avec une zone grise assymétrique ou symétrique voir dissymétrique
  - **HBsAg :** positif > 1,00

  Zone grise entre 0,9 et 0,99
  - **Toxo IgM :**positif > 1,20

  Zone grise entre 0,80 et 1,19
  - **CMV IgM :** positif > 1,20

  Zone grise entre 0,90 et 1,19
- Contrôles situés près du seuil: Ex :Syphilis avec BioRad Li ToRCH à 200 - 300

VITROS Syphilis Controls - Baseline Statistics [a][1]

Control[b]	Mean[c]	SD[d][2]
C1 [e][3]	0.00	0.147
C2 [f]	2.68	0.464

Particularités en Virologie/Sérologie

- Cas des contrôles positifs dilués (aHIV) avec variation des taux en fonction des lots de réactifs. Apport des panels de séroconversion
- Contrôles positifs uniquement près du seuil. Cas de suivi pour ToRC IgG
- Suivi de la population avec moyenne patients négatifs et pourcentage des patients en zone grise

Contrôles internes avec panels de séroconversion et échantillons dilués

QC Results

Parameter	Result	Release Limits
Calibrator Spread Limit	4%	≤ 16%
Calibrator Signal Index	-0.028	-0.500 to 0.300
C-A9 (Result)	0.16	≤ 0.30
C-B9 (Result)	3.5	≥1.9
C-C11 (Result)	4.6	≥2.8
C-D9 (Result)	74.5	≥ 50.0
C-C11/C-B9 Ratio	1.3	0.4 - 3.0

BBI HIV-1 Seroconversion Panel 952

Sample ID	*Vitros* Anti-HIV Assay		Acceptance Criteria Classification
	Result	Classification	
9521	0.16	Negative	Negative
9522	0.20	Negative	Negative
9523	0.41	Negative	Negative
9524	12.7	Reactive	Reactive
9525	44.3	Reactive	Reactive
9526	42.3	Reactive	Reactive

Contrôles internes avec panels de séroconversion et échantillons dilués

Verification Panel lot number: 120905

Part 2: IVDD Verification Panel:

QC results...

Parameter	Result	Release Limits
C-A9 (Result)	0.16	<= 0.30
C-B9 (Result)	3.53	>= 1.90
C-C11 (Result)	4.61	>= 2.80
C-D9 (Result)	74.5	>= 50.0
CAL1 %Spread	4	<= 16
Signal Index 1	-0.028	-0.500 - 0.300
Signal at Cutoff	200.767	N/A
C-C11/C-B9	1.31	0.40 - 3.00

For assay results, see page 1.

Panel Member	Result	Class	Acceptance criteria	Pass/Fail
P 1	3.30	Reactive	Reactive	Pass
P 2	1.46	Reactive	Reactive	Pass
P 3	1.17	Reactive	Borderline/Reactive	Pass
P 4	2.18	Reactive	Reactive	Pass
P 5	2.88	Reactive	Reactive	Pass
P 6	0.16	Negative	Negative	Pass

The data presented above represents a true summary of the release data for this batch.

Particularités en Virologie/Sérologie

I have compiled via econn Rubella IgM data from the last three months.
Amount of results is quiet significant:
6127 results on **43 systems** (3600 and 5600)
5168 patients results (84,3%) rest is control results
The distribution of the patient samples is as follow:

Reagent lot	Nbr of patients	negative	neg mean	%neg	Grey zone	% of Grey zone	Positive	%Positive
650	55	54	0.34	98.2	0	0.0	1	1.8
680	7			0.0		0.0		0.0
690	3			0.0		0.0		0.0
700	48	47	0.31	97.9	1	2.1	0	0.0
710	179	152	0.41	84.9	20	11.2	7	3.9
720	368	297	0.43	80.7	20	5.4	51	13.9
730	1849	1637	0.45	88.5	132	7.1	80	4.3
740	2218	1649	0.53	74.3	330	14.9	239	10.8
751	382	306	0.49	80.1	49	12.8	27	7.1
760	59	56	0.25	94.9	1	1.7	2	3.4

You will agree that there is a deviation in the negative patient mean as in the percentage of samples in the grey zone for lots **710,720,730,740 and 751**.

I find in my archives results from **1Q2012** that confirmed the "normal" distribution:

Total	4447	%	mean	median
Negative	4106	92.33	0.31	0.3
Grey Zone	94	2.11		

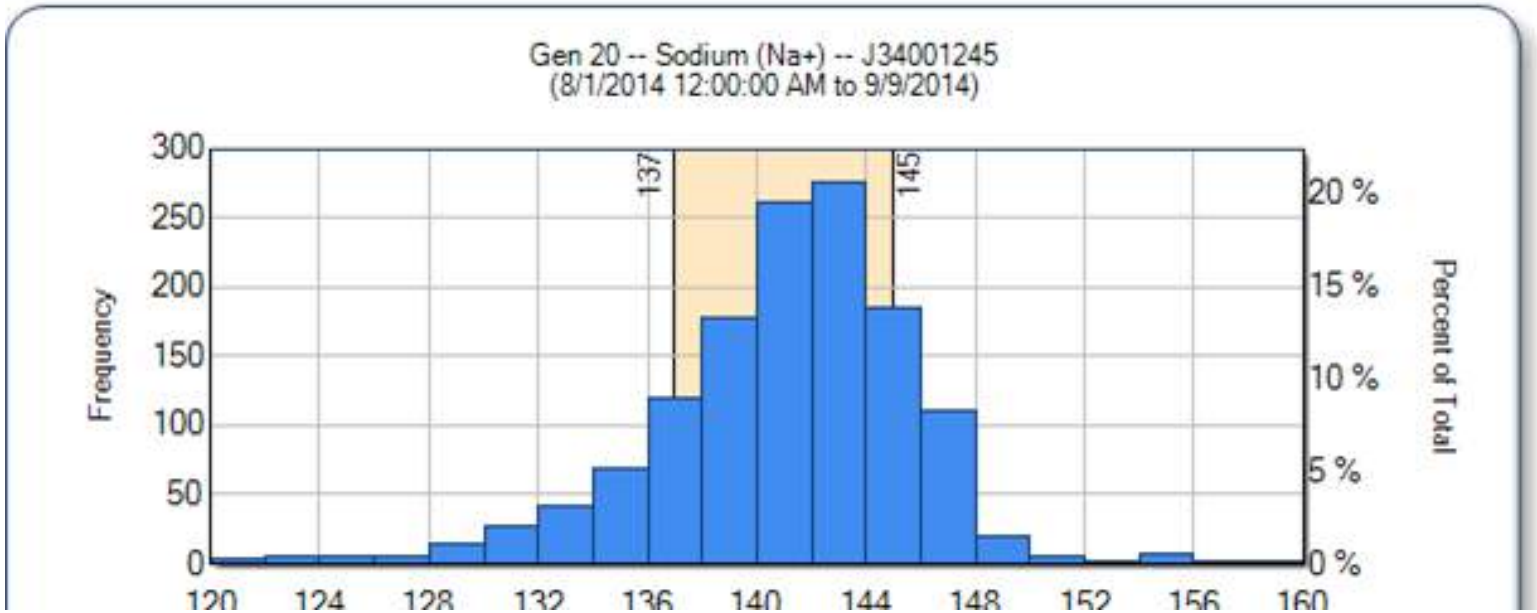
Syphilis TPA: data generated by econnectivity January 2012

		Percentage	Mean
Total samples	12956		
Total patients	12743	98.36	
Negative patients	12532	98.34	0.02
Grey zone	4	0.03	
Positive patients	207	1.62	

Exemple de moyenne patients

Patient Means -- Sodium (Na+) -- J34001245

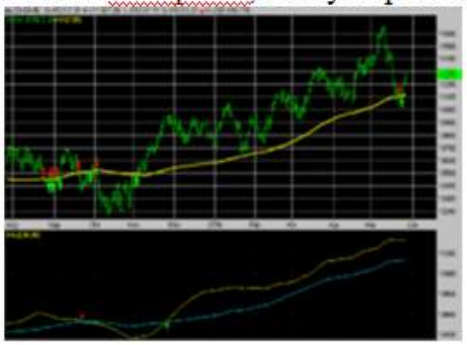
Gen	N	Mean	Median	Units	Percent Below Ref	Percent Above Ref
20	1377	140.32	141.37	mmol/L	19.2	16.2
22	5035	140.76	141.74	mmol/L	16.4	15
23	2431	138.35	139.04	mmol/L	30.6	2.3



Moyenne Mobile (Moving Average)

Moyenne glissante

Un article de Wikipédia, l'encyclopédie libre.



La **moyenne glissante**, ou **moyenne mobile**, est un type de moyenne statistique utilisée pour analyser des séries ordonnées de données, le plus souvent des séries temporelles, en supprimant les fluctuations transitoires de façon à en souligner les tendances à plus long terme. Cette moyenne est dite *mobile* parce qu'elle est recalculée de façon continue, en utilisant à chaque calcul un sous-ensemble d'éléments dans lequel un nouvel élément remplace le plus ancien ou s'ajoute au sous-ensemble.

Ce type de moyenne est utilisé généralement comme méthode de lissage de valeurs, en particulier dans le domaine financier pour l'analyse technique de cours boursiers.

How Moving Averages Can Help Enhance Quality Control and Improve your Laboratory

June 18, 2014

DATA INNOVATIONS
Simple Ideas, Better Solutions

Moving Averages in the Lab – What does it do?

- "Normalizes" result data so that the lab can gauge the likelihood that a trend will continue
- Proactively monitors instrument stability between QC cycles in the background
- Enables preemptive intervention before the process fails by detecting shifts, trends & momentum
- Uses Error and Warning Thresholds to automatically push notifications to key laboratory staff, and in conjunction with auto-verification allows for a true "walk away" process

Value of Moving Average / Moving Medians

- Value…

Instantly and automatically detect and notify when analytical errors occur without increasing operational costs.

- How?
  - ✓…By continuously monitoring results production
  - ✓…That detects analytical errors days before traditional QC,
  - ✓…Using revenue generating samples

Moving Averages Compliments QC

Standard QC

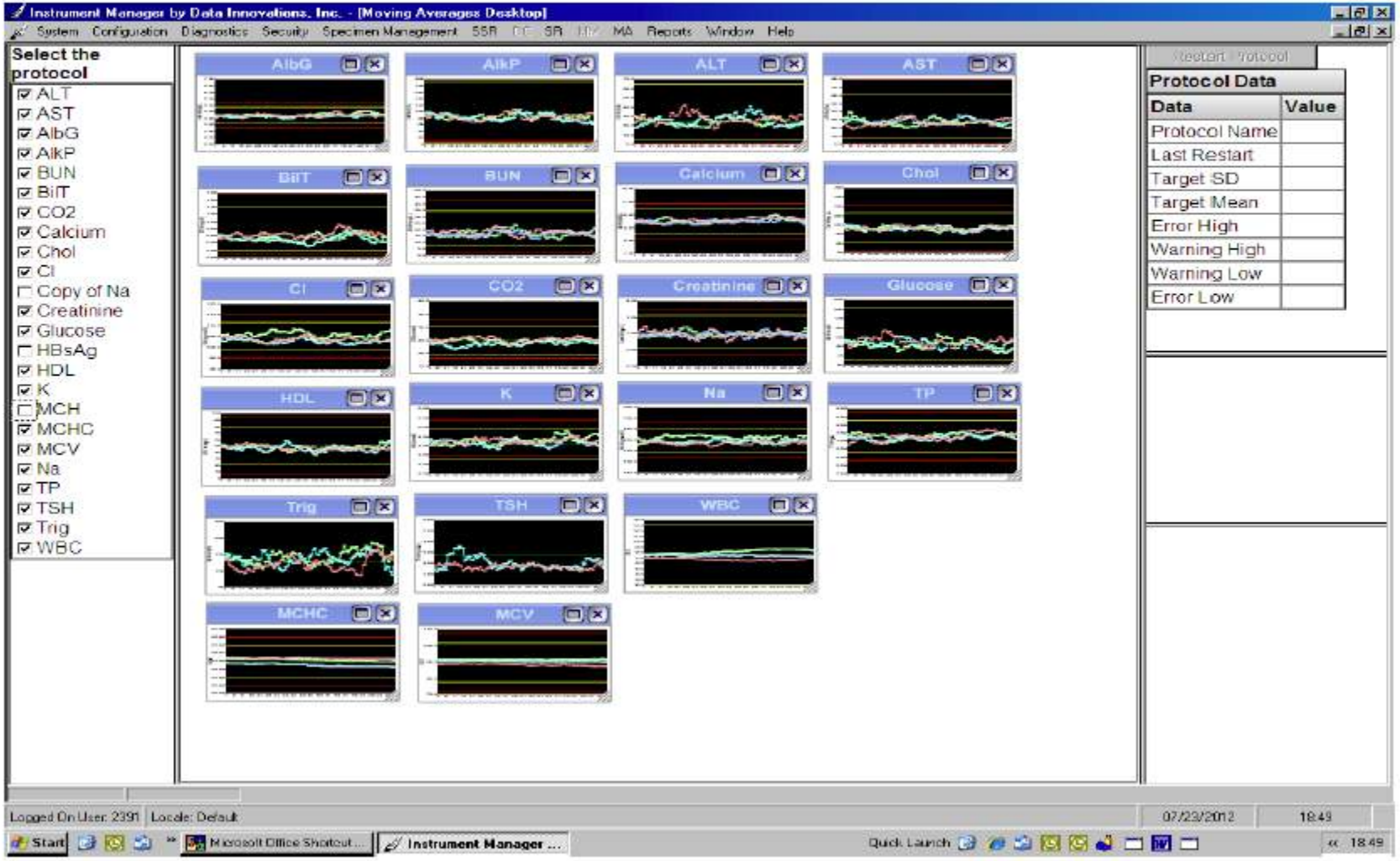
- QC is a "snapshot" in time
- Usually performed post maintenance & calibration
- Potential for hours (or days) before some errors are detected
  - ✓ Once a shift – 60-70% of test volume between 6 am to 11 am or
  - ✓ Once per day (100% of test volume before next data point
- Matrix Effects

Moving Averages

- Real-Time, proactive process providing continuous monitoring using Patient Samples
  - ✓ Early detection of shifts and drifts hours / days before traditional QC
    - Continuous data points to detect shifts/drifts
- Automatically "pushes" instrument status stability notifications
  - ✓ Provides data points, while producing revenue generating activities
    - No Instrument out of production
    - No Dedicated resource (walk away) with notification capabilities
    - No non-reimbursed reagent material or control material
- QC can be run at recommended regulatory intervals (Cost savings in $ and time)

Moving Averages Desktop Example

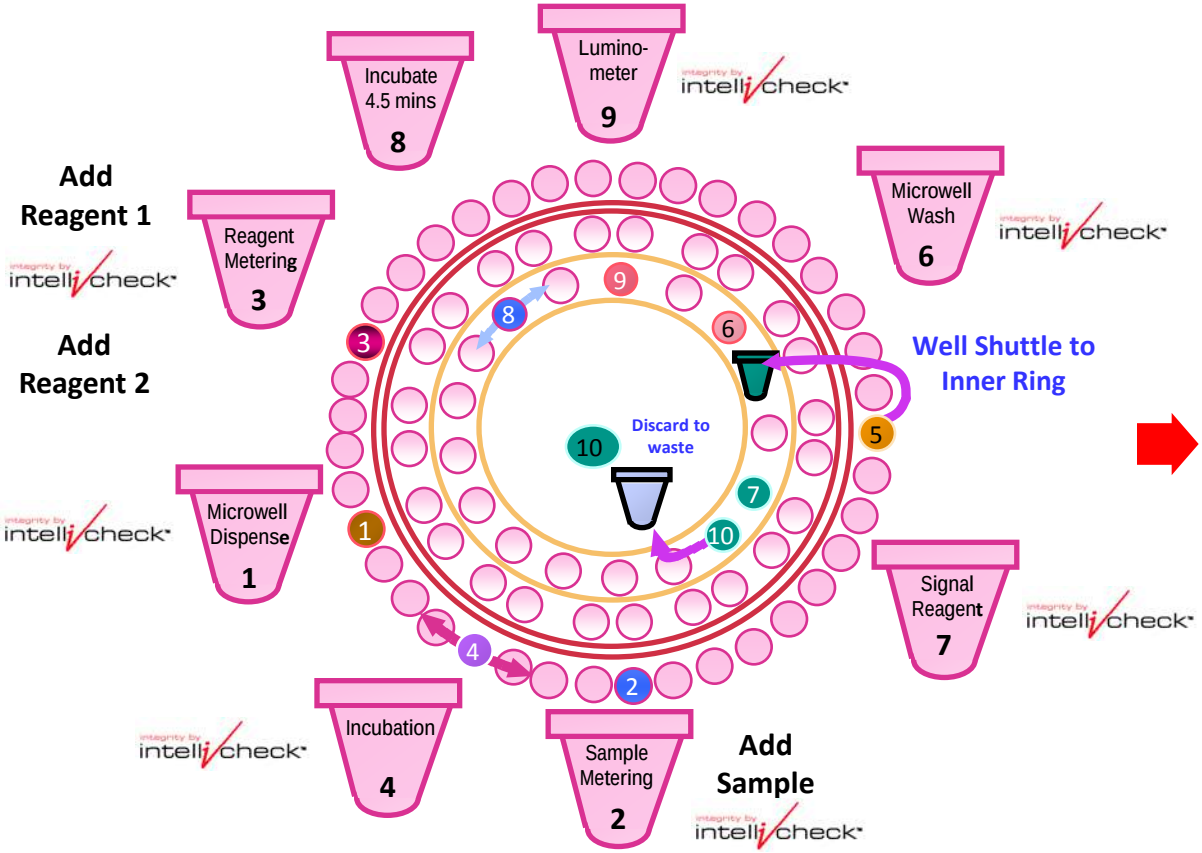
Screenshot from customer monitoring results in real-time



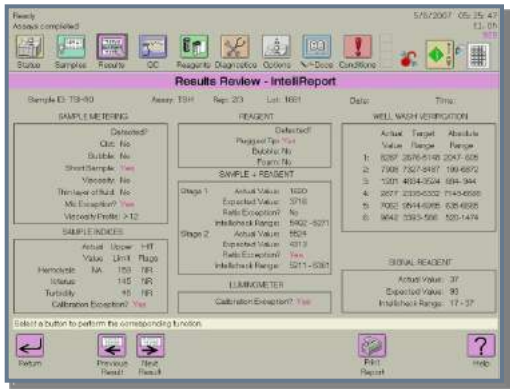
VITROS® Immunodiagnostic System 3600

Ortho Clinical Diagnostics
a Johnson & Johnson company

Intellicheck® Technology

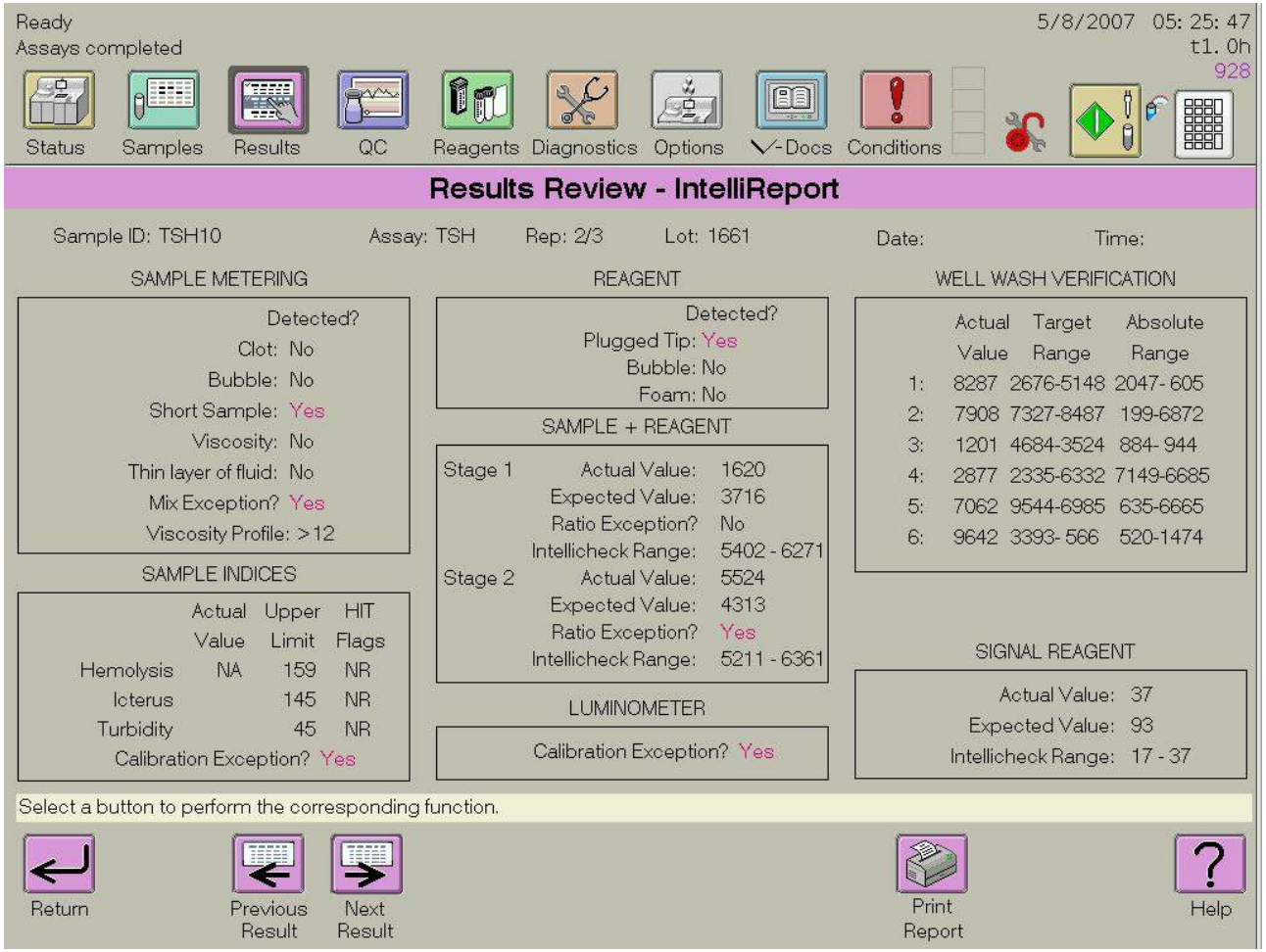


IntelliReport™



Traceability of all results with both real-time and retrospective verification and documentation of quality for every result

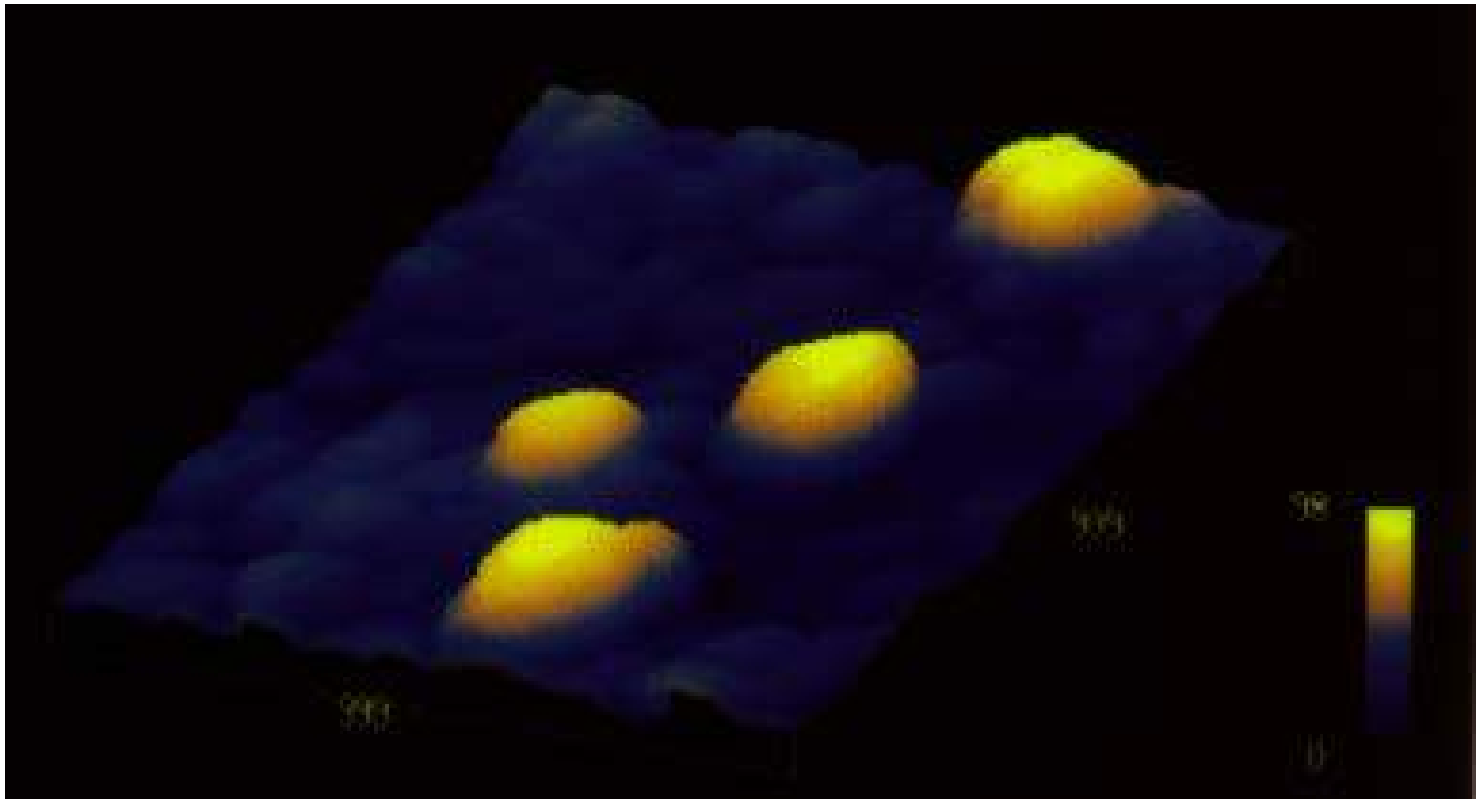
IntelliReport



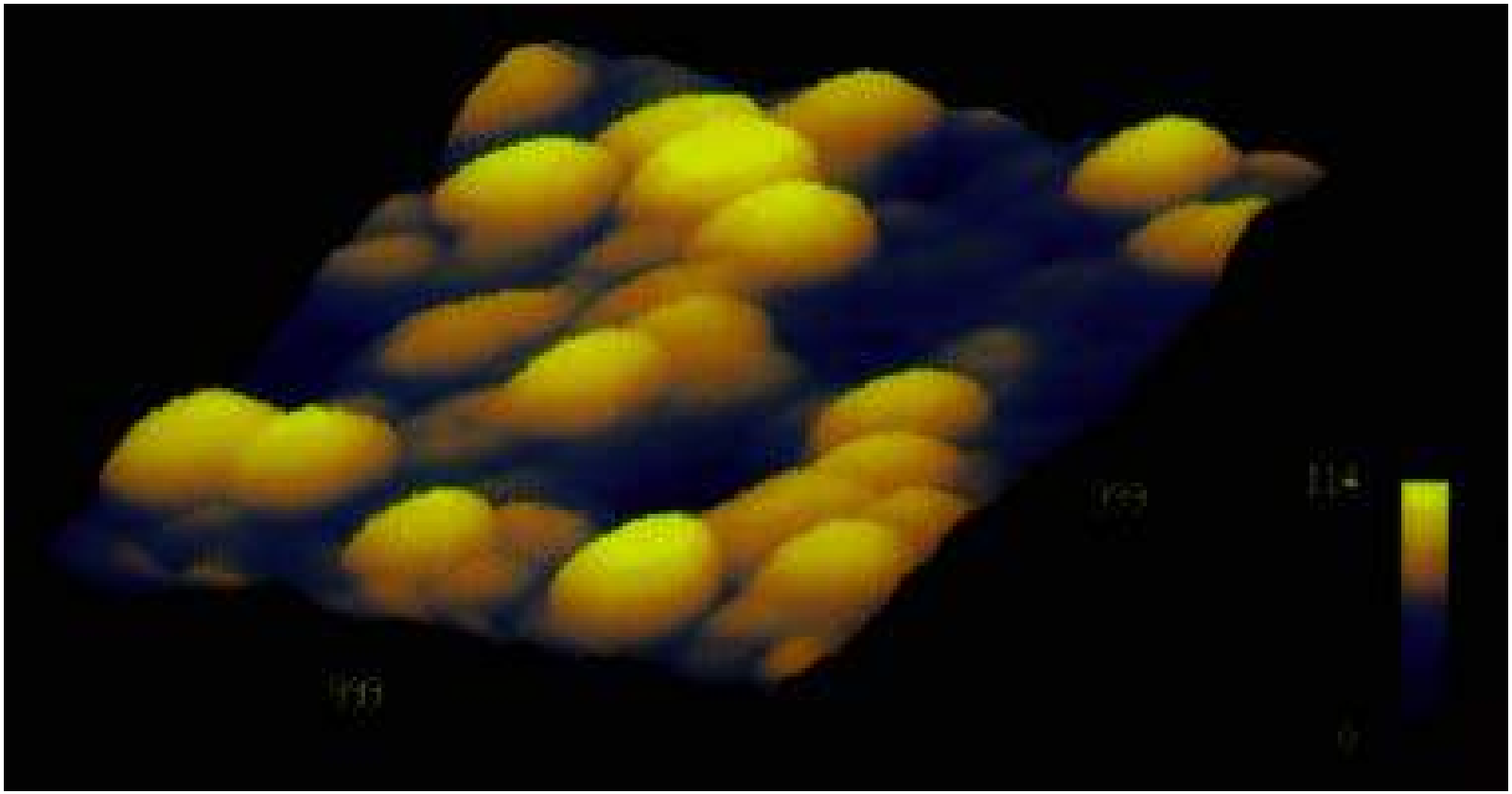
Merci pour votre attention

Questions??

Ferritin Antibody: Passive Adsorption



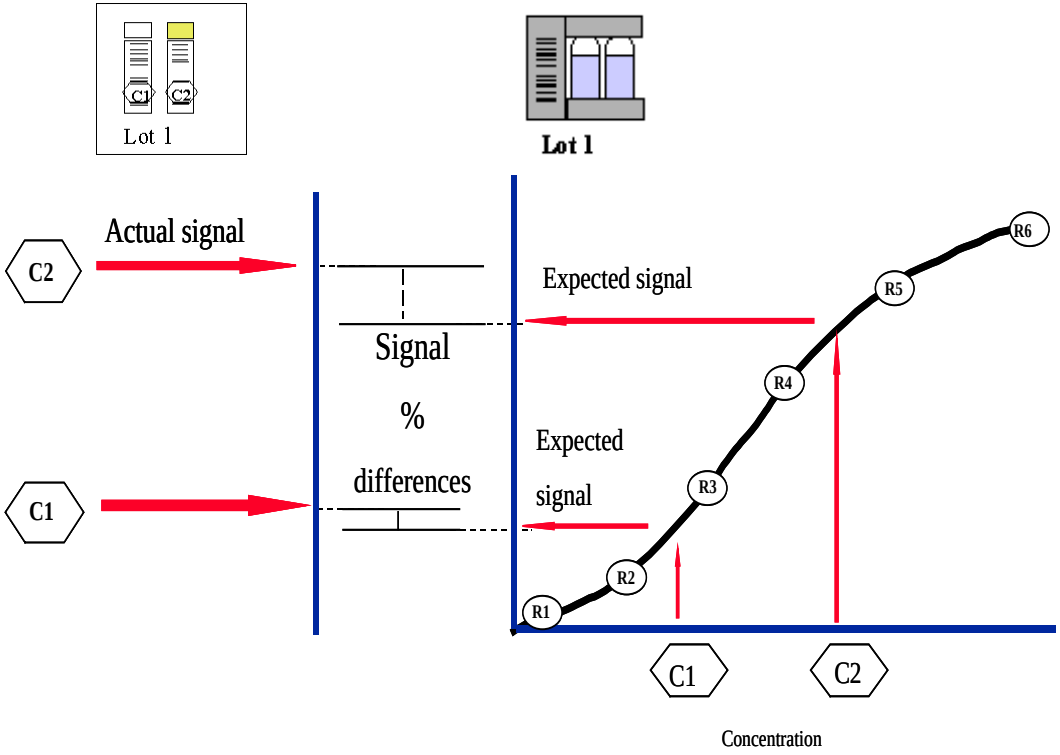
Ferritin Antibody: Biotinylated on *Vitros* Streptavidin Coated Well

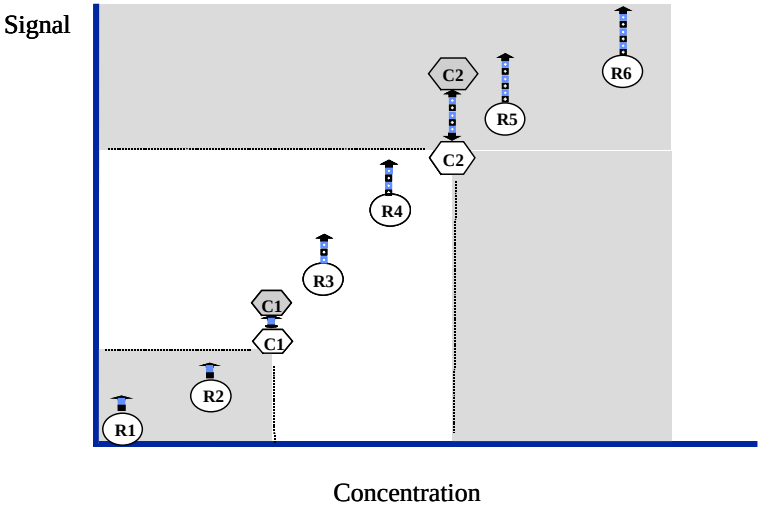


Quantitative assays: calibration theory

At Customer site: the lot specific calibrators are run and the signal measured.

Master Calibration Curve is rescaled using the Customer Calibrators actual readings





Biotin Interference

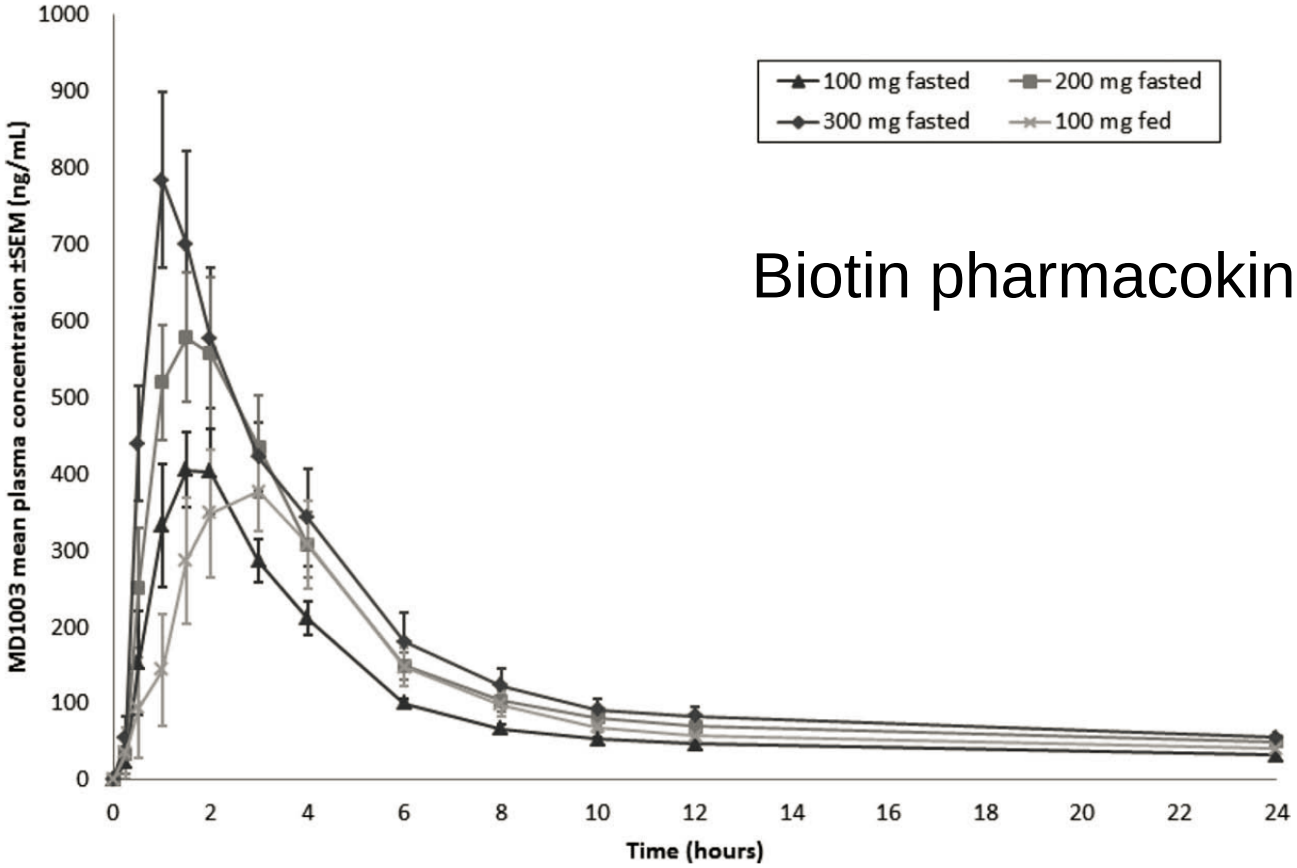
- Biotin is also known as Vitamin B_7, a.k.a. Vitamin H
- Readily available OTC as a nutritional supplement with health and beauty claims
- Most clinicians are neutral on biotin supplements. Low biotin levels are very rare.

Dietary Requirements*:

- **30 µg** (123 nmol) of Biotin per day is defined as an **adequate** intake for adults.
- The normal, diet-derived biotin intake in Western populations has been estimated to be 35 to 70µg/d (143–287 nmol/d)
- Consumer demand is a recent phenomenon and continues to rise. Supplements often contain very high doses of biotin.
- When IVD assays were designed, expected levels of biotin were quite low. Now that biotin supplements are becoming more popular, the likelihood of encountering higher levels has increased.

*The Journal of Nutritional Biochemistry 1999 Mar; 10(3)

Biotin interference



Biotin pharmacokinetics

Laure Peyro Saint Paul, Danièle Debruyne, Delphine Bernard, Donald M. Mock & Gilles L. Defer (2016) Pharmacokinetics and pharmacodynamics of MD1003 (high-dose biotin) in the treatment of progressive multiple sclerosis, Expert Opinion on Drug Metabolism & Toxicology, 12:3, 327-344, DOI: 10.1517/17425255.2016.1136288

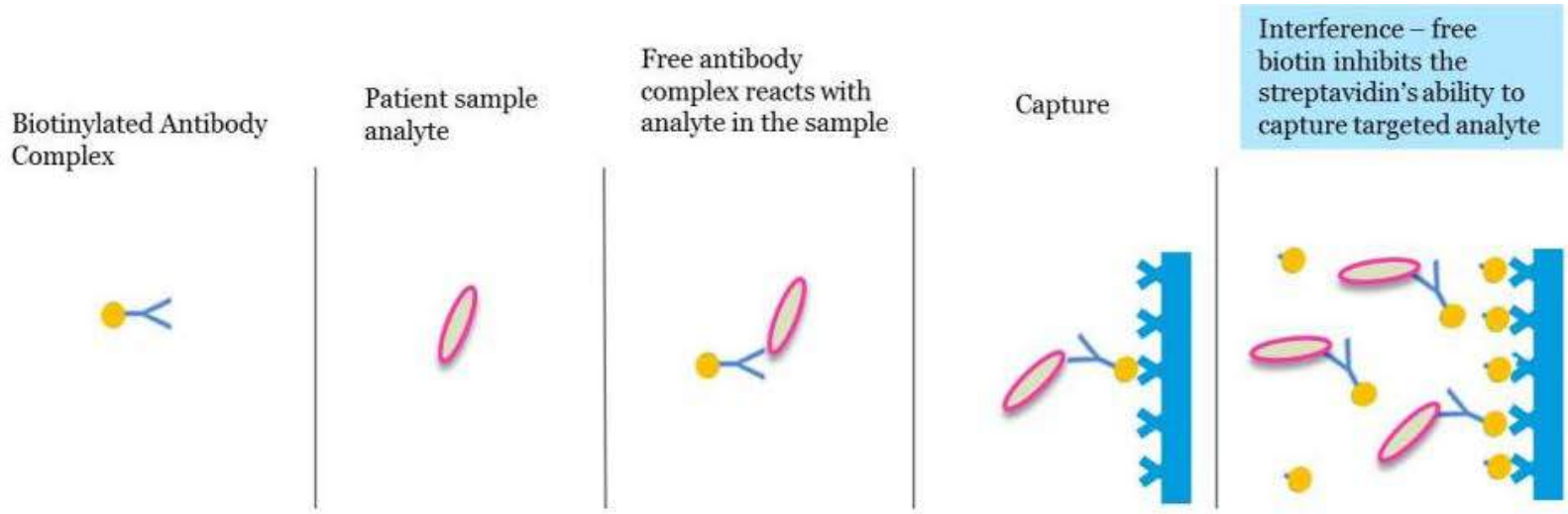
Biotin interference

Competitive immunoassays: excess biotin in the specimen competes with the biotinylated analog for the binding sites on streptavidin, resulting in the potential for a **false positive.**

Sandwich immunoassays: excess biotin in the sample displaced biotinylated antibodies, resulting in the potential for a **falsely lower results.**

Interference generally correlates positively to sample volume (e.g. aHBe 80 µL) and correlates negatively to well binding capacity (e.g. E2 and Testo).

Only SAC wells are potentially affected, but not all assays are affected equally.



Biotin interference

Sample drawn	RESULTS					
	E2 (pg/mL)	FSH (mIU/mL)	FT3 (pmol/L)	TT4 (nmol/L)	Testo (ng/dL)	TSH (mIU/L)
21/10/2014	2401	8.3	3.7	7.2	608	<0.015
31/10/2014	21	54.1	2.5	7.1	16	1.22

ANALYTE	PRE	1HR	2HR	3HR	4HR	5HR	22HR	RR	ASSAY TYPE
TSH (mIU/L)	2.06	0.05	0.03	0.1	0.21	0.24	3	0.47-4.68	SANDWICH

Biotin Interference

- **It's not just Ortho** who has interfering substances with its immunoassays, it's industry-wide, every IVD manufacturer is impacted.
- **It's not just biotin**, there are various substances that could potentially interfere with IA performance (e.g. hemolysis, serum proteins, HAMA, autoantibodies, drugs like acetaminophen). Labs are constantly vigilant against interferences. CLSI guidance puts responsibility for this vigilance on the lab.
- Biotin interference, due to consumption patterns, is a recent development and **Ortho is responding.** Ortho has always and will continue to manage interfering substances in development (e.g. new tests tPSA II and iPTH are not affected). Some assays will be reformulated ("overcoating") to reduce interference. IFUs will be updated to explicit biotin interference at different biotin concentrations. A customer communication will be issued in Jan 2018.

VITROS® 3600 Immunodiagnostic System Menu[1,2]

Cardiology

CK-MB
Troponin I ES
Myoglobin
NT-proBNP

Thyroid

Free T4
Free T3
Total T4
Total T3
TSH 3rd Gen
T3 Uptake

Metabolic

Cortisol (serum & urine)
NTx
Intact PTH**

Endocrine

Total β-hCG II
Estradiol
Progesterone
Testosterone
LH
FSH
Prolactin

Anemia

Ferritin
B12
Folate
RBC Folate

Infectious Disease[3]

*HBsAg ES**
HBsAg, Confirm+
Anti-HBs
Anti-HCV+
Anti-HBc+
Anti-HBc IgM+
*HBeAg**
*Anti-HBe**
Anti-HAV Total
Anti-HAV IgM
Anti-HIV 1+2+
HIV Ag/Ab Combo**
*Toxoplasma IgG**
*Toxoplasma IgM**
Rubella IgG
*Rubella IgM**
*CMV IgG**
*CMV IgM**
Syphilis

Oncology[4]

Total PSA~
CEA
AFP
CA 125 II™
CA 15-3™
CA 19-9™

Prenatal

*AFP**

[1] Product availability subject to local regulatory requirements.

[2] Assays in **bold** are available; Unless noted otherwise, the remainder of the menu (*italics*) is available within 6 months of launch depending on validation and regulatory requirements. In U.S., Hepatitis B, C, and HIV require supplemental PMA submission.

[3] Hepatitis and HIV co-developed with Novartis Vaccines and Diagnostics, Inc.

[4] PSA, CA125 II, CA 15-3, CA 19-9 are trademarks of Fujirebio Diagnostics, Inc.

+ Available outside U.S. February 2009

~Available Q1 2009

* Not approved or cleared for U.S. Market

** In Development

January 2009

The LIAISON® family collection menu

Leading position in Specialty Assays

AUTOIMMUNITY
ANA Screen[2]
dsDNA[2]
tTG IgA[2]
ENA Screen[2]
Cardiolipin IgG[2]
Cardiolipin IgM[2]

BONE & MINERAL
25-OH Vitamin D TOTAL
N-TACT® PTH Gen II
1-84 PTH
Osteocalcin
BAP OSTASE®
1,25 dihydroxyvitamin D[1]
FGF 23**
Sclerostin**

CARDIAC MARKERS
Troponin I[2]
Myoglobin[2]
CK-MB[2]

ENDOCRINOLOGY
THYROID
TSH (3rd Gen.)
Free T3
Free T4
T3
T4
Tg
Anti-Tg
Anti-TPO

Estradiol
hCG/ß-hCG
DHEA-S

ADRENAL FUNCTION
ACTH
Cortisol

GROWTH
hGH
IGF-I

DIABETES
C-Peptide
Insulin

HYPERTENSION
Direct Renin
Aldosterone

INFECTIOUS DISEASE
SEPSIS
SEPSIS BRAHMS PCT®[2]
BRAHMS PCT® II Gen

CHAGAS
Chagas IgG

TREPONEMA
Treponema Screen

EBV
EBV IgM
VCA IgG
EBNA IgG
EA IgG

TORCH
CMV IgG Avidity
HSV-1/2 IgG
HSV-1 IgG
HSV-2 IgG
HSV-1/2 IgM
Parvovirus B19 IgG
Parvovirus B19 IgM

BORRELIA
Borrelia burgdorferi IgG
Borrelia burgdorferi IgM

VZV
VZV IgG
VZV IgM

MYCOPLASMA
Mycoplasma pneumoniae IgG
Mycoplasma pneumoniae IgM

MEASLES & MUMPS
Measles IgG
Measles IgM
Mumps IgG
Mumps IgM

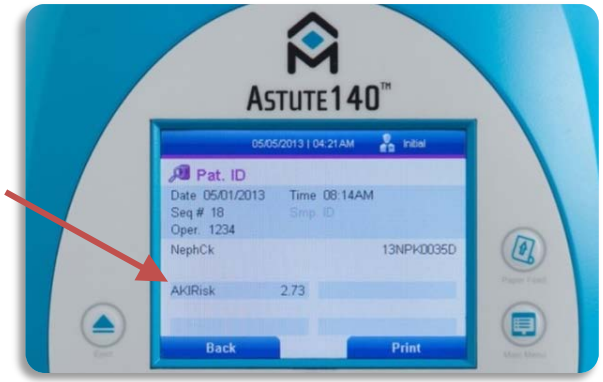
CHLAMYDIA
Chlamydia T. IgG
Chlamydia T. IgA

BORDETELLA
Bordetella pertussis Toxin IgG
Bordetella pertussis Toxin IgA

VIRAL HEPATITIS AND RETROVIRUSES

The NEPHROCHECK® Test Results

The ASTUTE140® Meter automatically calculates and displays a single numerical test result.



The NEPHROCHECK® Test Result (AKIRISK™ Score) is displayed on the ASTUTE140® Meter screen without units after the NEPHROCHECK® Test procedure is completed. Results for the individual markers are not displayed.

The single numerical NEPHROCHECK® Test Result (AKIRISK™ Score) is calculated by multiplying the concentrations of the two biomarkers, and then dividing by 1000:

$$\text{The NEPHROCHECK® Test Result (AKIRISK™ Score)} = \frac{[\text{TIMP-2}] * [\text{IGFBP-7}]}{1000} \quad (\text{units} = (\text{ng/ml})^2/1000)$$

NephroCheck®

VITROS® Technologies & Analyseurs

Results matter.

System	VITROS® Technologies
VITROS® 5600 *Integrated System*	125 assays
VITROS® 4600 *Chemistry System (VITROS 5,1 FS)*	75 assays
VITROS® 3600 *Immunodiagnostic System*	50 assays
VITROS® ECi/ECiQ *Immunodiagnostic System*	50 assays
VITROS® 350 *Chemistry Systems*	43 assays

Biorad QC results Toxo G

Assay	Liquicheck ToRCH Positive control
Bayer ADVIA Centaur	27.0 IU/ml
Abbott AxSYM	19.8 IU/ml
Beckman Access	19.9 IU/ml
BioMerieux Vidas	18.0 IU/ml
VITROS	**16.8 IU/ml**
Abbott IMx	16.7 IU/ml
DPC Immulite	14.7 IU/ml
Diasorin LIAISON	14.6 IU/ml
Roche COBAS	14.2 IU/ml
Diasorin ETI RUBEK-G	13.8 IU/ml
DPC Immulite 2000	13.5 IU/ml