

Current Status and New Recommendations for HbA1c Testing

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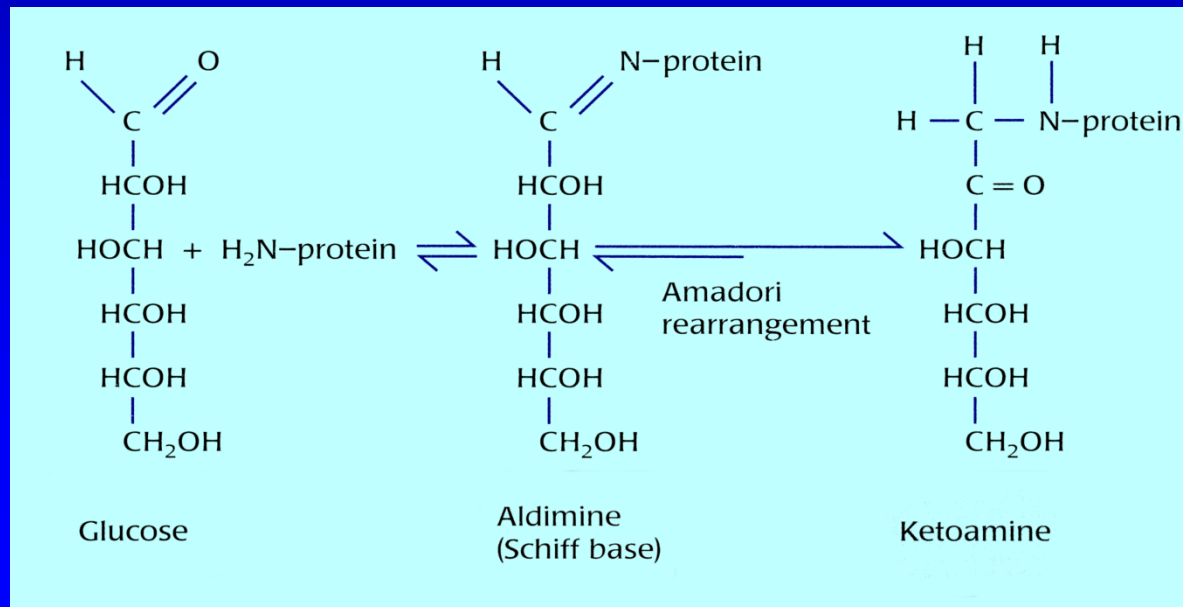


HbA1c Testing

- HbA1c Background:
 - What is it?
 - How is it measured?
- Standardization: Why and How?
- Current status of reporting HbA1c
- HbA1c for diabetes diagnosis
- Improving measurement of HbA1c

What is it?

Glycated hemoglobins are stable minor hemoglobin components formed slowly and nonenzymatically from hemoglobin and glucose.

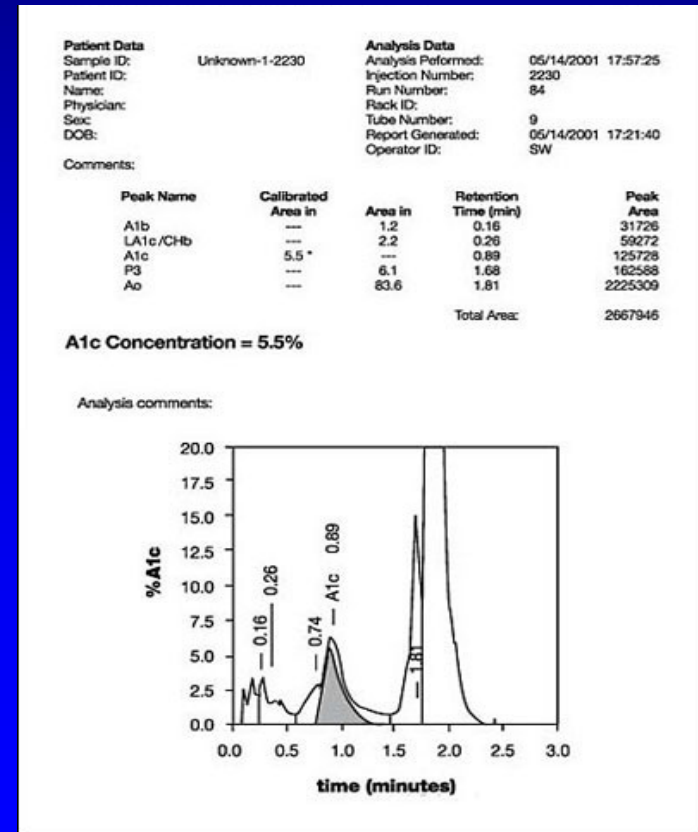
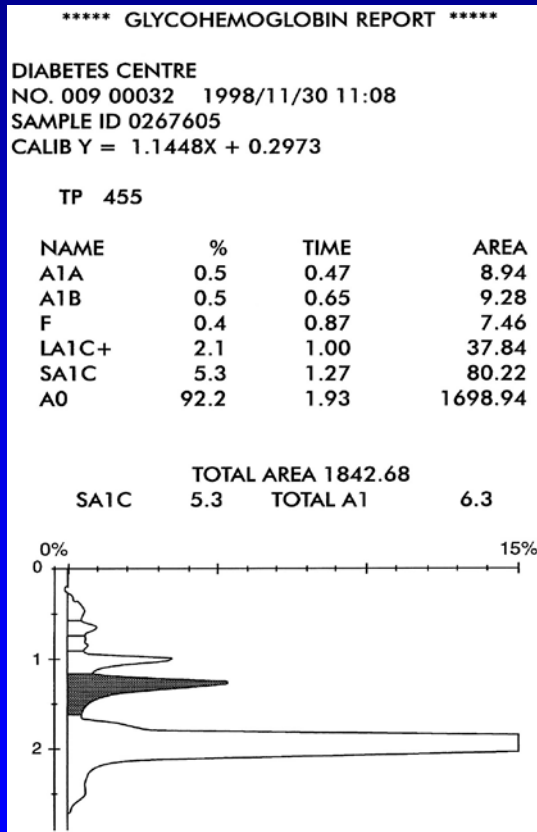


How is it Measured?

- Cation-exchange chromatography (HbA1c)
- Affinity binding/chromatography (total GHB)
- Immunoassay (HbA1c)

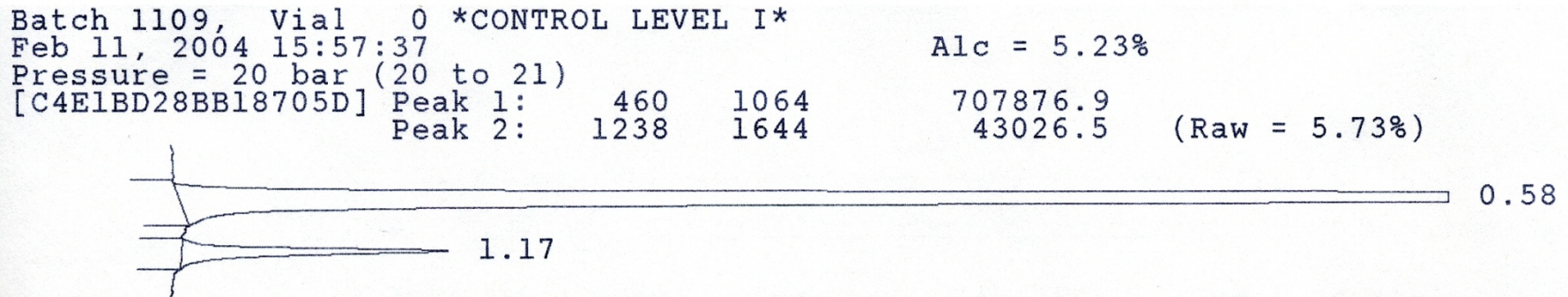
Cation-exchange Chromatography:

- Separation is achieved by utilizing differences in ionic interactions between the cation exchange group on the column resin surface and the hemoglobin components in the sample.



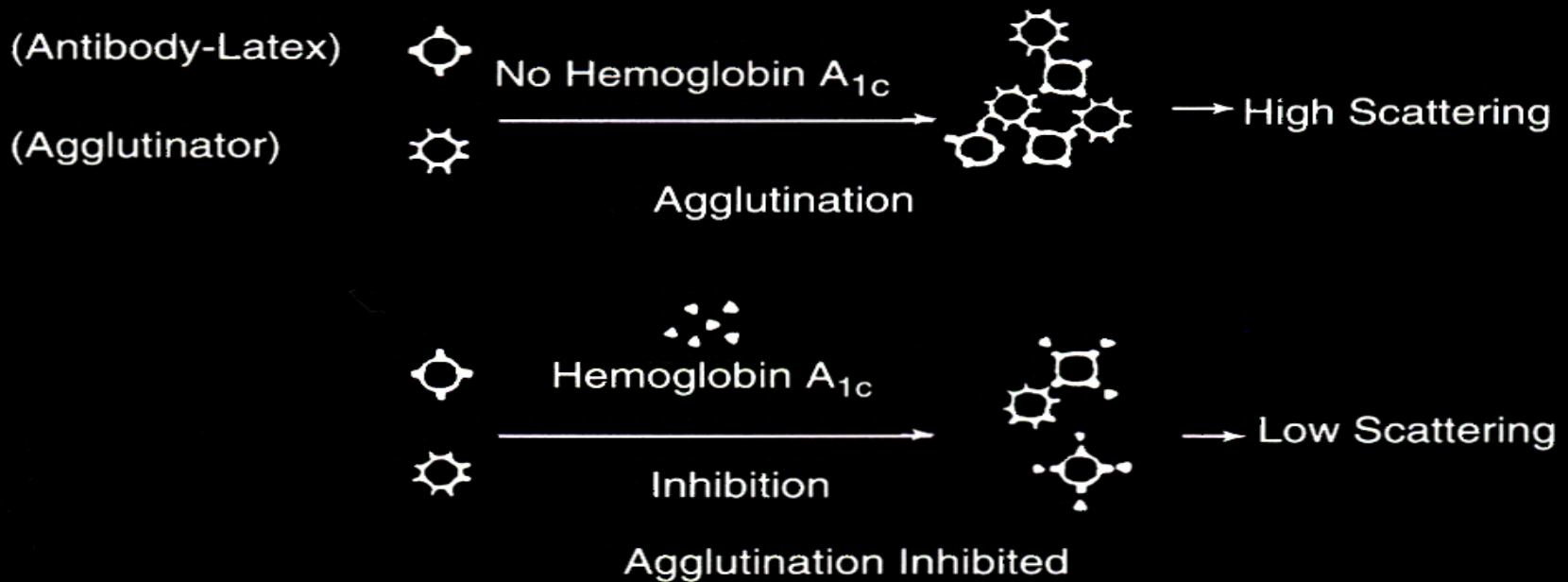
Boronate Affinity Chromatography:

- Boronic acid reacts with the cis-diol groups of glucose bound to hemoglobin
- Non-glycated hemoglobin does not bind to the column and is eluted first



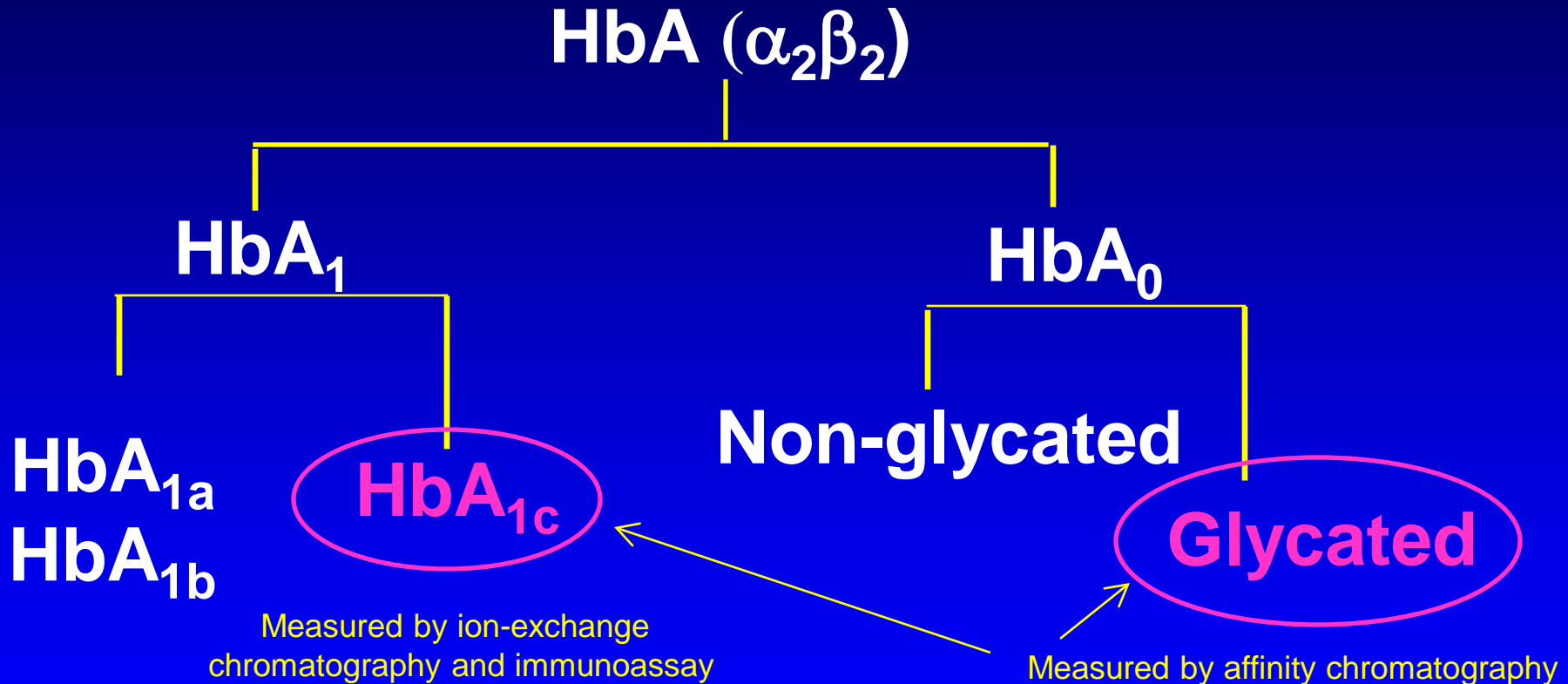
Immunoassay

Inhibition Of Latex Agglutination



- An agglutinator causes agglutination of Ab- coated latex particles, measured by increased turbidity.
- HbA_{1c} in the specimen reacts with the Ab causing inhibition of the agglutination measured by a decrease in turbidity.

Glycated Hemoglobin



Standardization: Why and How?

Diabetes Control and Complications Trial (DCCT) (1983-1993)

- Are the vascular complications of diabetes related to glycemic control?
- Can intensive treatment decrease long-term complications?
- 1440 patients studied over 10 years

DCCT: glucose and HbA1c

	Intensive	Conventional
Blood Glucose mmol/L (mg/dL)	8.6 (155)	12.8 (231)
HbA1c %	7.2	9.1

1993 - DCCT results reported:

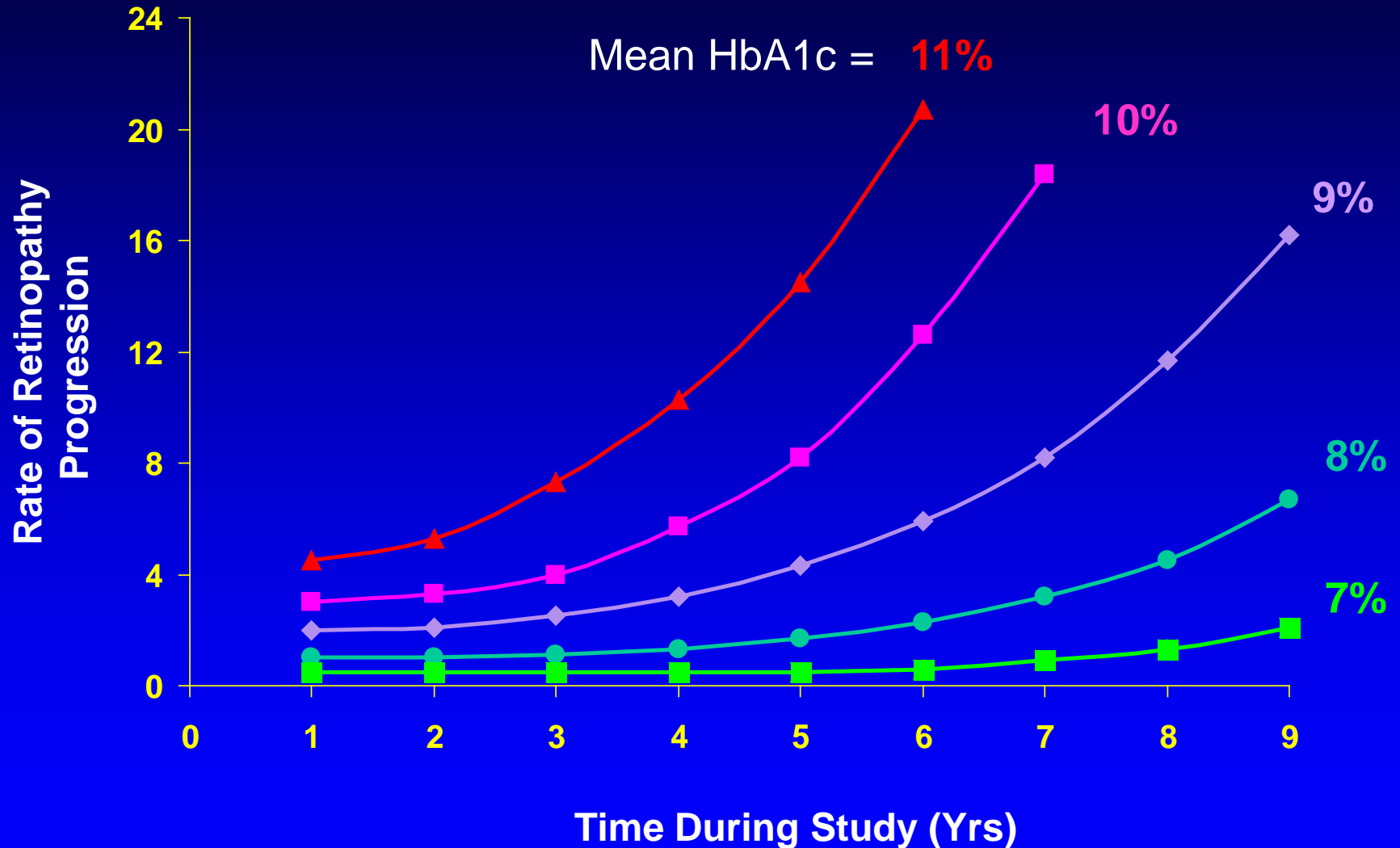
Intensive therapy reduced:

- retinopathy by 34-76%
- microalbuminuria by 35%
- clinical albuminuria by 56%
- clinical neuropathy by 60%

DCCT :

For Type 1 diabetes, the degree of glycemic control (measured as HbA1c) was closely related to risk for development and/or progression of chronic diabetic complications

HbA1c and the Risk of Retinopathy in the DCCT



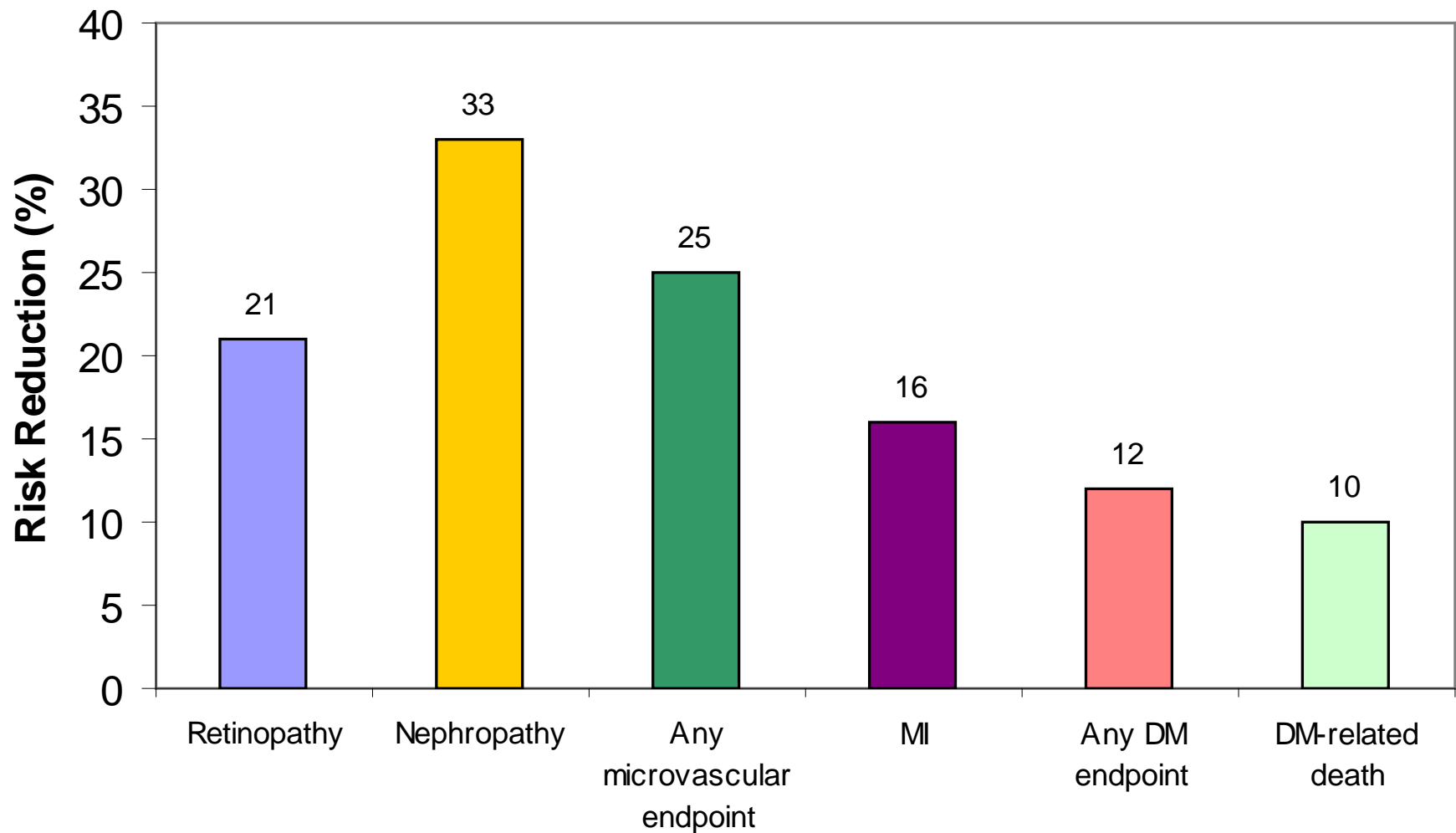
DCCT

The DCCT set the stage for establishment of specific diabetes treatment goals using HbA1c as an index of mean glycemia.

United Kingdom Prospective Diabetes Study (UKPDS)

Provided conclusive evidence for a decreased risk of diabetic complications with intensive control of blood glucose in patients with Type 2 diabetes

United Kingdom Prospective Diabetes Study (UKPDS)

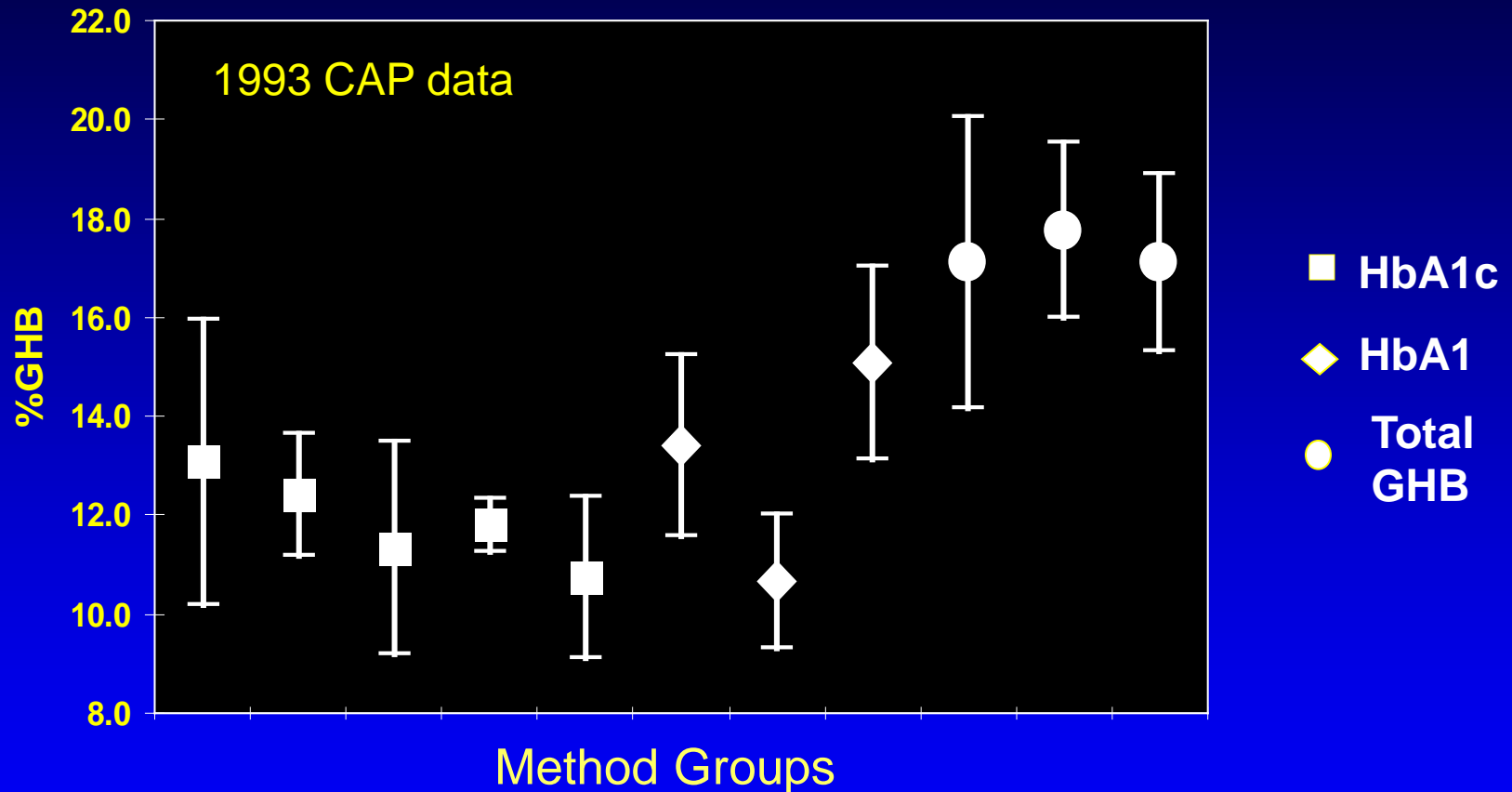


American Diabetes Association (ADA) Recommendations 1994-2011

	<u>Goal</u>
A1C	<7%*

* Referenced to a non-diabetic range of 4.0-6.0%
using a DCCT-based assay

BUT – what did HbA1c results look like at the end of the DCCT?



- In 1993, CAP data showed that there was a lot of variability among methods measuring different glycated Hb fractions.
- Clearly this was not acceptable for achieving specific HbA1c goals.

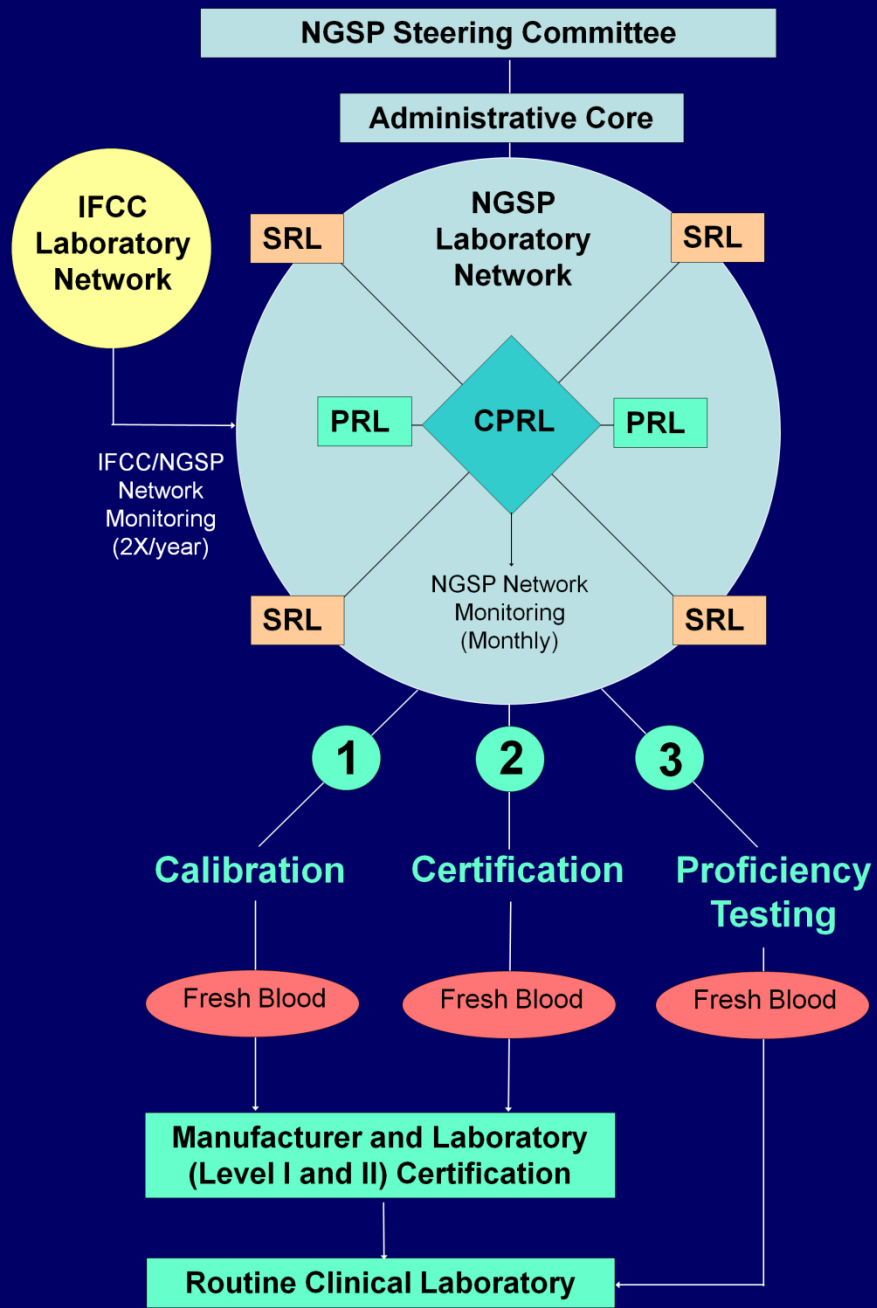
HbA1c: From Chaos to Order

- Early studies showed that different method types could produce equivalent results when calibrated to a common reference.
- In 1993, the AACCC formed a Subcommittee on Glycated Hemoglobin Standardization with the goal of standardizing assay methods to the DCCT reference method.
- In 1996, the National Glycohemoglobin Standardization Program (NGSP) was formed to implement the AACCC program.



www.ngsp.org

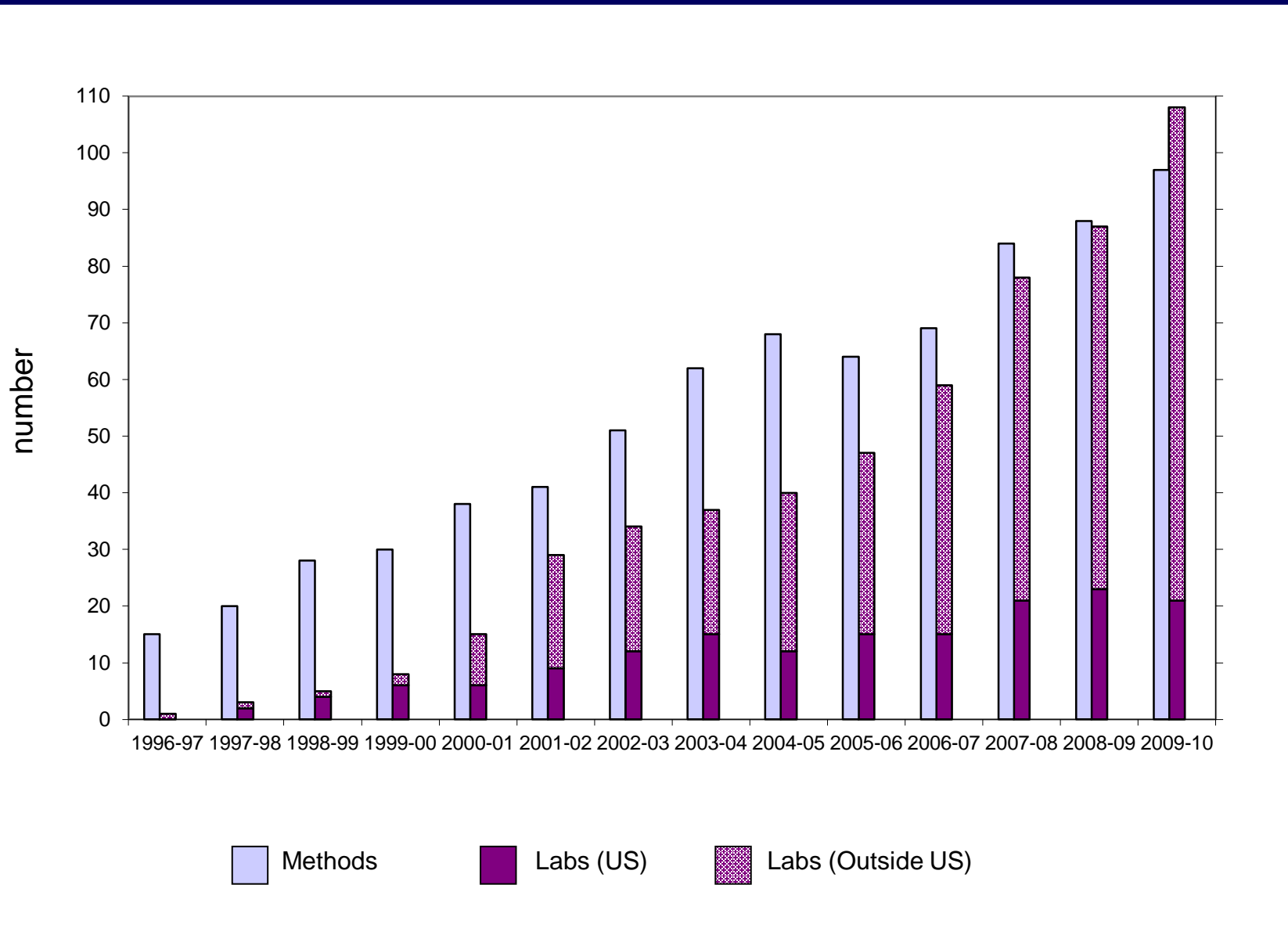
Purpose: to standardize HbA1c test results to those of the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) which established the direct relationships between HbA1c levels and outcome risks in patients with diabetes.



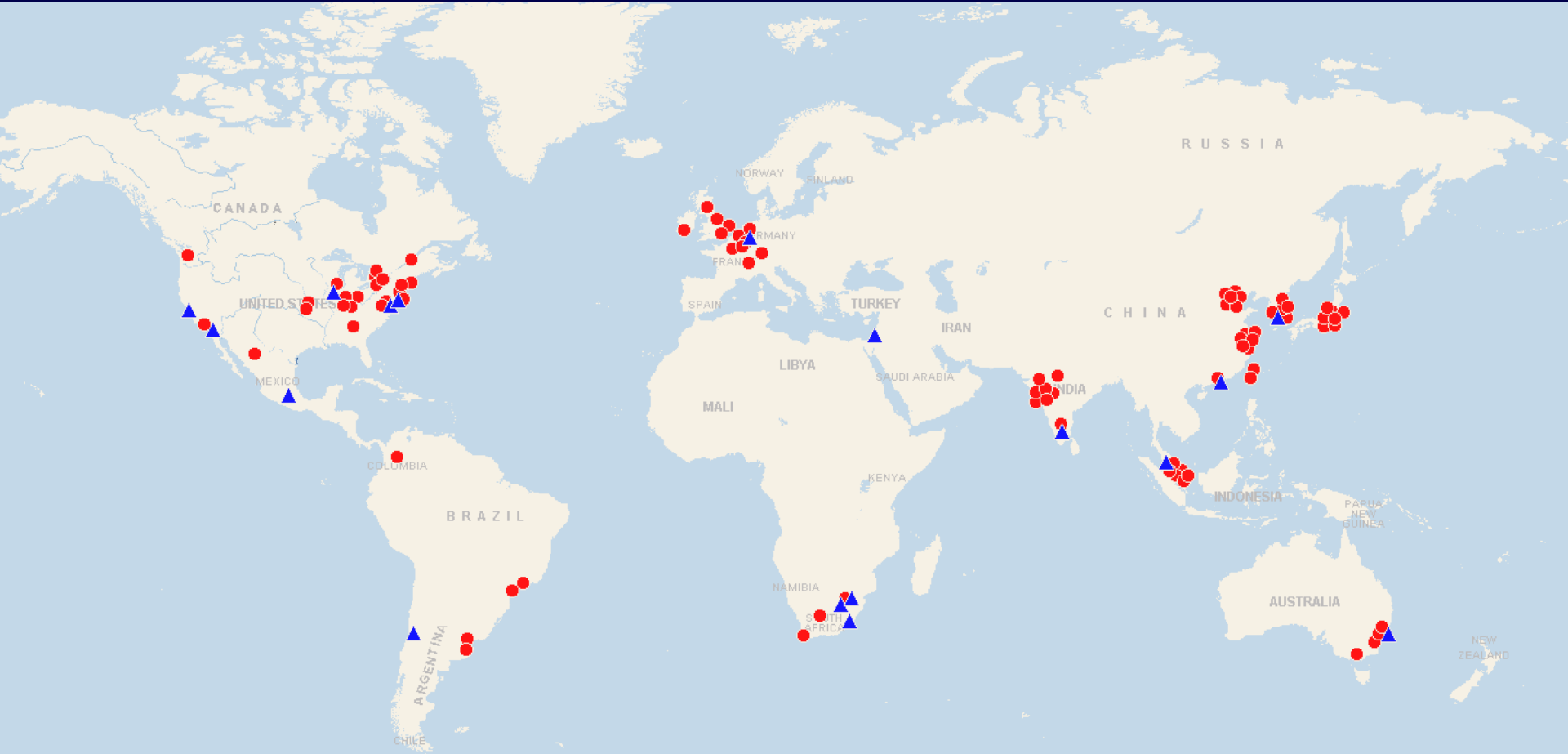
NGSP Certification

Certification Type	# samples compared	Bias Criteria	Monitoring (yes / no)	Monitoring Protocol
Manufacturer	40	95% CI of differences within $\pm 0.75\%$ GHB	No	-
Level I Lab	40	95% CI of differences within $\pm 0.70\%$ GHB	Yes	10 Samples Quarterly
Level II Lab	40	95% CI of differences within $\pm 0.75\%$ GHB	No	-

Increase in Certification: Year 1 to 14



Distribution of NGSP Certified Laboratories (2/11)

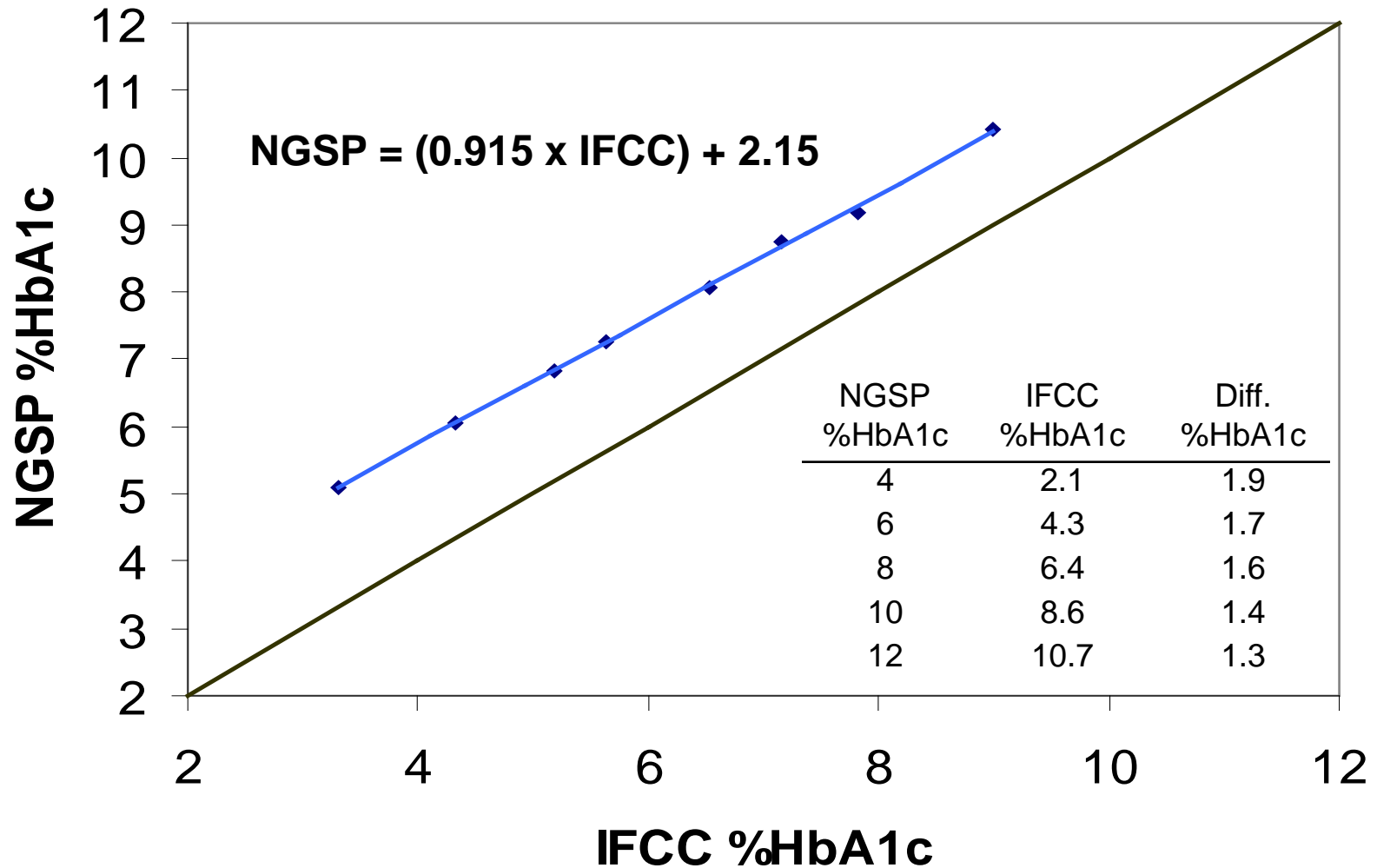


● Level 1 Labs ▲ Level 2 Labs

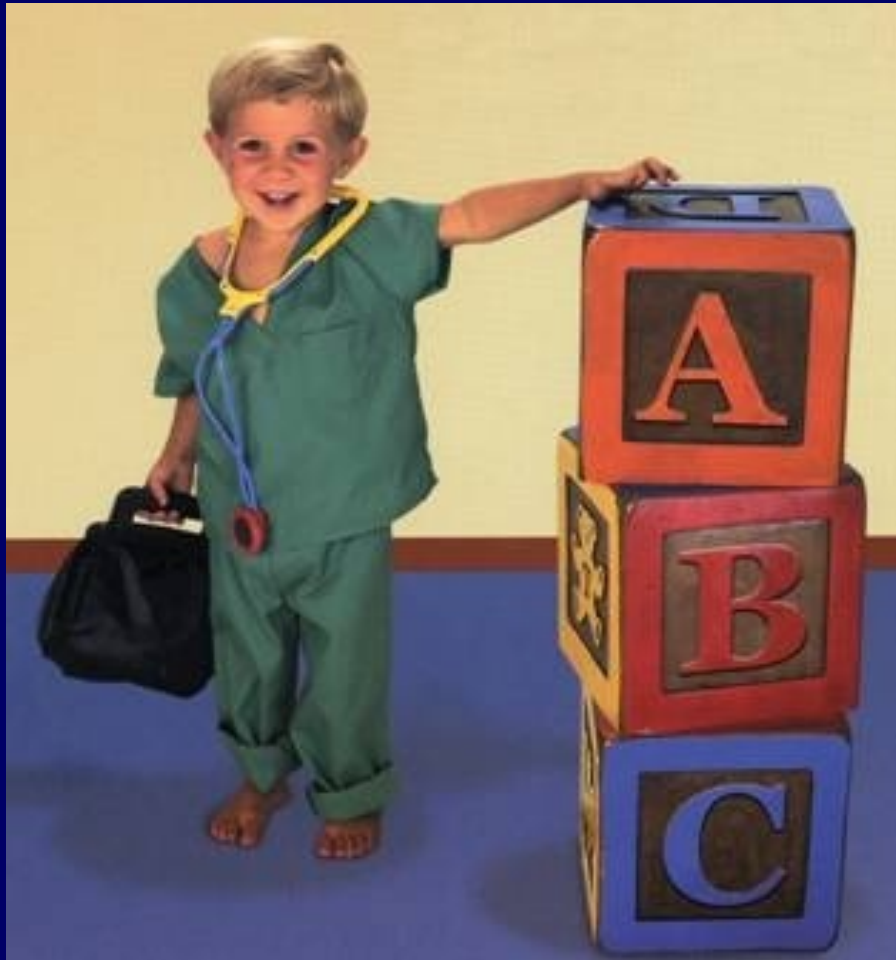
IFCC Standardization

- **1995-2001:** IFCC Reference Method (higher order method) developed and approved; results show linear relationship with NGSP but were 1.3 to almost 2% lower than DCCT equivalent results

Which Numbers to Report?

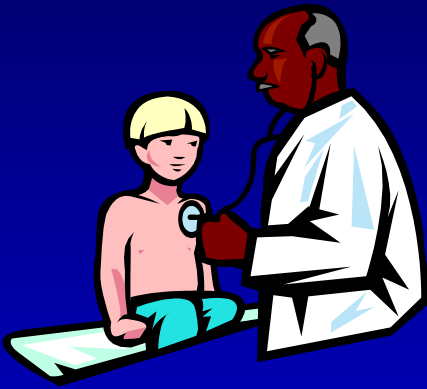


HbA1c results in a lab report



- HbA1c = 6.5%
- Is his blood sugar normal or elevated?
- Is his diabetes under control or out of control?
- That depends on which number scale is being used - NGSP or IFCC

The Balance



Patient Care



Traceability

Standardization and Reporting

- 2004: A master equation was established between the NGSP and IFCC network results:

$$\text{NGSP} = 0.915 * \text{IFCC} + 2.15$$

- ❖ Linear equations were also developed to describe the relationship between IFCC and the standardization schemes in Japan and Sweden
- ❖ These relationships are monitored on a regular basis to ensure traceability

Standardization and Reporting

- **2004:** ADA, EASD, IDF met to discuss the controversy in reporting units for HbA1c. They considered the possibility of reporting HbA1c as a mean blood glucose and recommended a study of mean glucose vs. HbA1c.

Standardization and Reporting

- **2007: IFCC / IDF/ EASD / ADA Consensus Statement**
 - ❖ HbA1c test results should be standardized worldwide to the IFCC Reference system
 - ❖ A1C results are to be reported world-wide in IFCC units (mmol/mol) and NGSP units (%).
 - ❖ If the “average plasma glucose study” fulfills it’s a *priori* specified criteria, an A1C-derived average glucose (ADAG) value calculated from the A1C result will also be reported as an interpretation of the A1C results.

Standardization and Reporting

- **2008:** The ADAG (A1c derived average glucose) study showed a linear relationship between HbA1c and average glucose and recommended reporting of estimated average glucose (eAG), derived from HbA1c, as an educational tool.

HbA1c vs. Mean Blood Glucose

A1C assay and estimated average glucose values

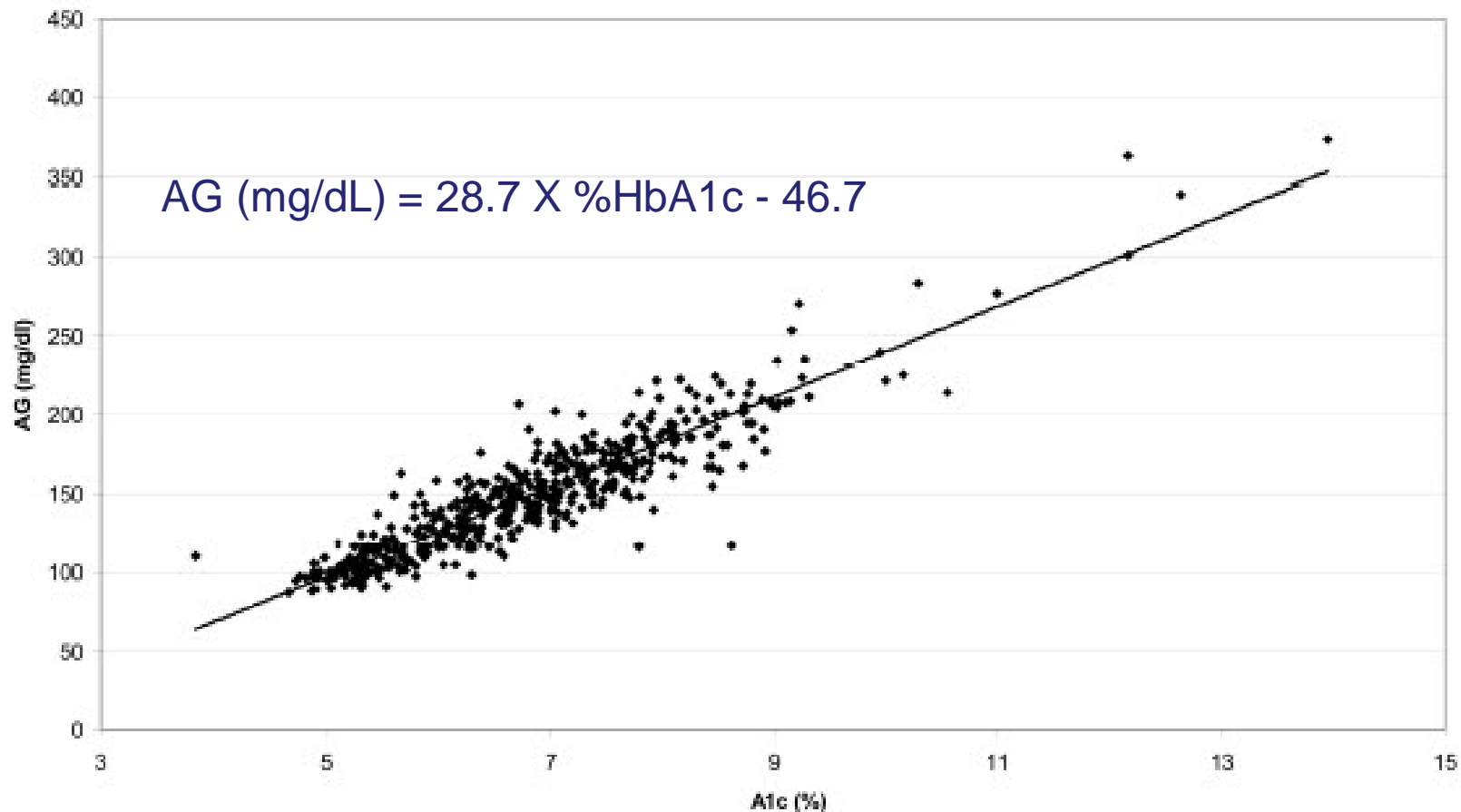


Figure 1—Linear regression of A1C at the end of month 3 and calculated AG during the preceding 3 months. Calculated $AG_{mg/dl} = 28.7 \times A1C - 46.7$ ($AG_{mmol} = 1.59 \times A1C - 2.59$) ($R^2 = 0.84$, $P < 0.0001$).

Standardization and Reporting

- **2010:** Another consensus statement on the Worldwide Standardization of the HbA1c (ADA, EASD, IFCC, ISPAD)
 - ❖ HbA1c results are to be reported in SI units (mmol/mol) and NGSP units (%)
 - ❖ Both results (IFCC and NGSP) should be reported in manuscripts

Current Status of HbA1c Reporting (1)

- Officially, there is worldwide consensus that HbA1c should be reported in both NGSP (%) and IFCC (mmol/mol) units.
- However, the decision on what to report is still being made country by country.

Current Status of HbA1c Reporting (2)

- The US will continue to report NGSP %HbA1c. Reporting of eAG has also been recommended by the ADA and AACC but will be calculated and reported by the laboratory (US only).
- Most other countries have decided to change to IFCC numbers – most will dual report for at least 1-2 years :
 - ❖ UK
 - ❖ Ireland
 - ❖ The Netherlands
 - ❖ Scandinavia
 - ❖ Italy
 - ❖ Australia
 - ❖ New Zealand
 - ❖ Germany
 - ❖ Canada
- Japan will likely continue to report JDS and NGSP %

Current Status of HbA1c Reporting (3)

- Although the world will again be reporting different numbers, results will be traceable to IFCC numbers as well as to clinical data through linear equations that are carefully monitored.
- All relevant journals will require reporting in both units.

HbA1c for Diabetes Diagnosis

2009 American Diabetes Association Recommendations for Diagnosis

- Diagnostic cutoffs:
 - ❖ Fasting glucose ≥ 126 mg/dL or
 - ❖ 2-h PG ≥ 200 mg/dL or
 - ❖ Symptoms with casual PG > 200 mg/dL
- Fasting glucose ≥ 100 mg/dL and < 126 is diagnostic for *impaired fasting glucose*.

International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes

THE INTERNATIONAL EXPERT COMMITTEE*

An International Expert Committee with members appointed by the ADA, EASD and IDF was convened in 2008 to consider the current and future means of diagnosing diabetes in non-pregnant persons. The report of the International Committee represents the consensus view of its members and not necessarily the view of the organizations that appointed them.

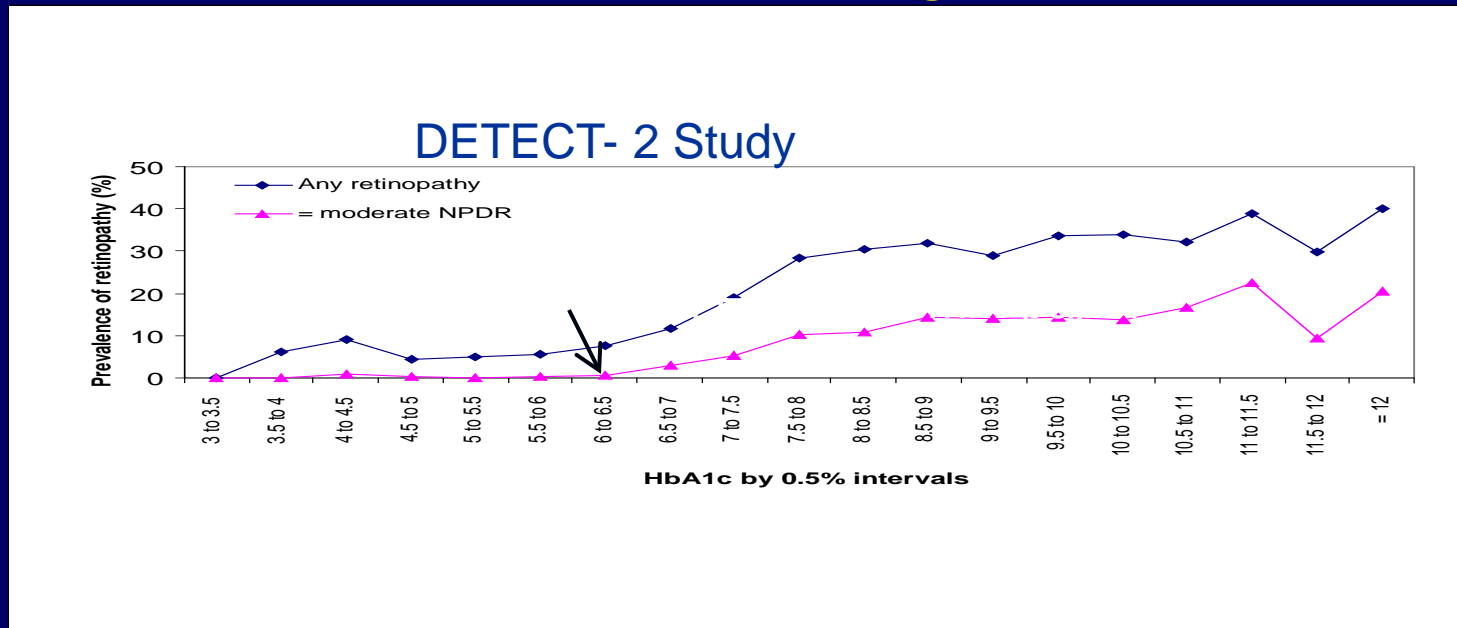
Advantages of HbA1c Compared to Glucose: 2009 International Expert Committee Report

- Standardized and aligned to the DCCT/UKPDS; measurement of glucose is less well standardized
- Better index of overall glycemic exposure and risk for long-term complications
- Substantially less biologic variability
- Substantially less preanalytic instability
- No need for fasting or timed samples
- Relatively unaffected by acute perturbations in glucose levels
- Currently used to guide management and adjust therapy

Limitations of HbA1c Compared to Glucose: 2009 International Expert Committee Report

- Interferences from certain conditions
 - Hb variants
 - Conditions that affect red cell turnover
- High cost and/or lack of availability in some parts of the world

HbA1c Cut Point for the Diagnosis of Diabetes



- Data from Detect-2 show that the level at which the prevalence of diabetes-specific “moderate” retinopathy begins to rise is at 6.5% HbA1c.
- Among those with HbA1c <6.5%, “moderate” retinopathy was virtually nonexistent.

International Expert Committee Report Recommendations

- The diagnosis of diabetes is made if the HbA1c level is $>6.5\%$. Diagnosis should be confirmed with a repeat HbA1c test unless clinical symptoms and glucose levels >200 mg/dL are present.
- In parts of the world where HbA1c testing is not available or is inadequate, or if HbA1c testing is not possible due to factors interfering with its measurement, clinicians should continue to use the previously recommended approaches to diagnose diabetes based on glucose measurements.

International Expert Committee Report Recommendations (cont'd)

- Individuals with an HbA1c level $\geq 6\%$ but $< 6.5\%$ are likely at the highest risk for progression to diabetes.
- HbA1c tests to diagnose diabetes should be performed using clinical laboratory equipment. Point-of-care instruments have not yet been shown to be sufficiently accurate or precise for diagnosing diabetes.

2010 ADA Recommendations:

Criteria for Diagnosis of Diabetes

1. HbA1c \geq 6.5%

OR

2. FPG \geq 126mg/dl

OR

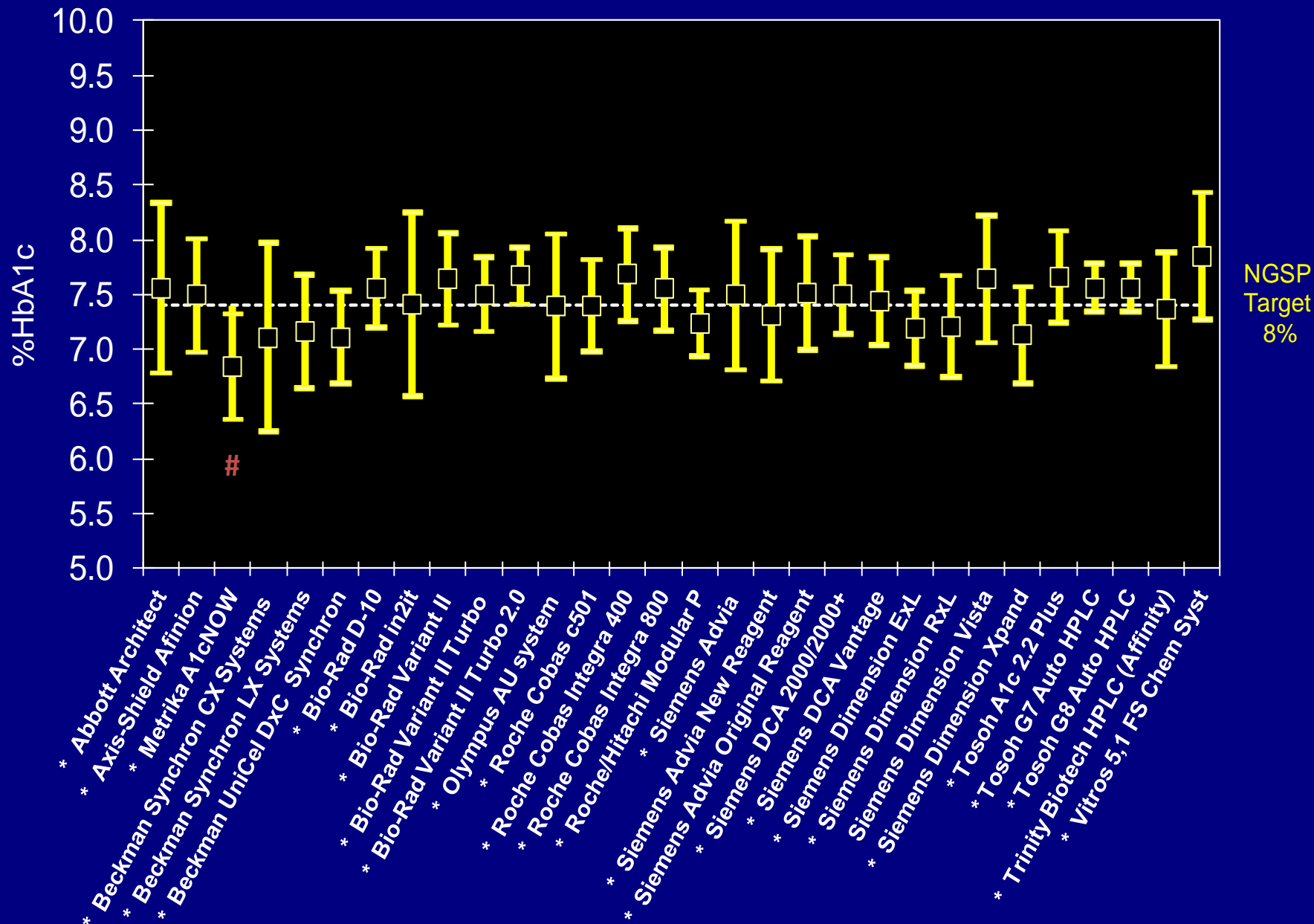
3. Two-hour plasma glucose \geq 200 mg/dl during a 75g OGTT

OR

4. Random glucose \geq 200 mg/dl in a patient with classic symptoms of hyperglycemia

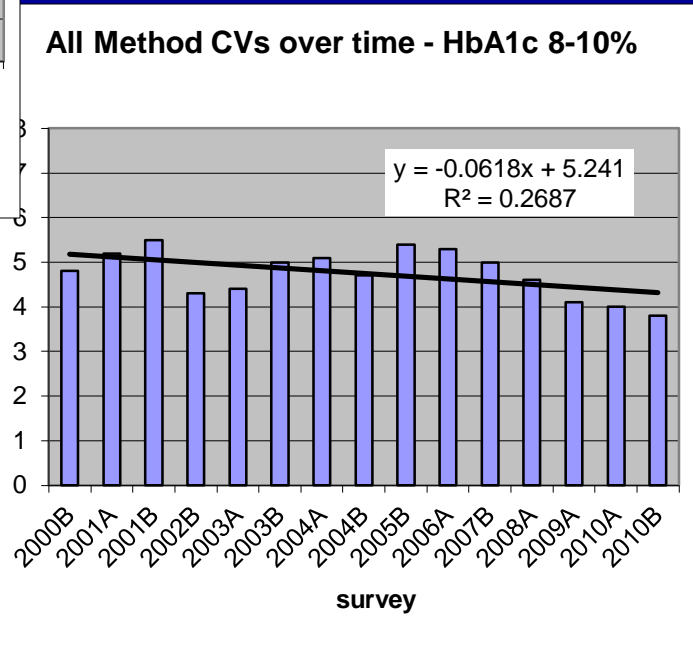
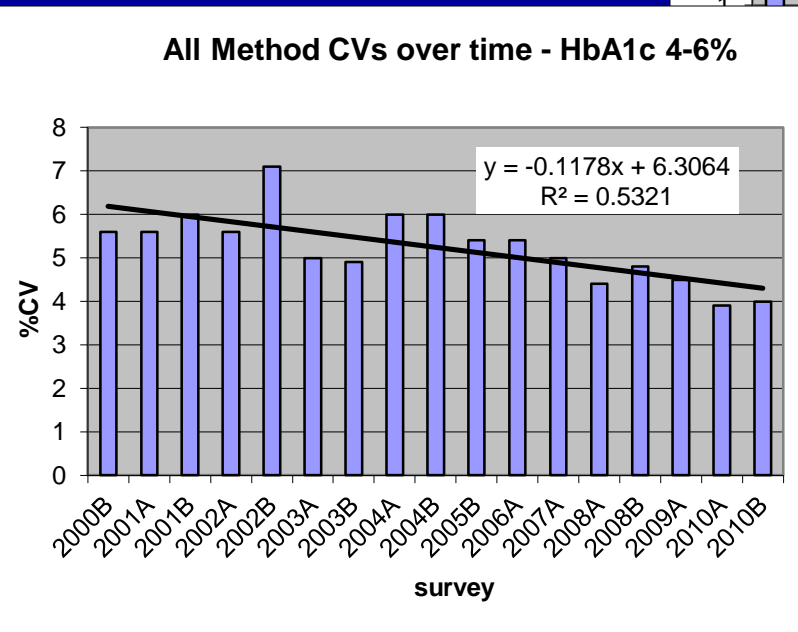
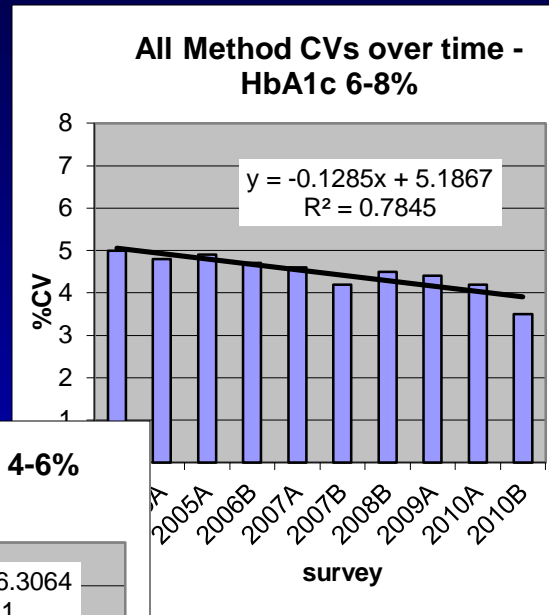
Improving measurement of HbA1c

CAP GH2-A 2010 mid level (mean \pm 2SD)



EDTA interference

Decrease in All-Method CVs Over Time 2000-2010



Improving HbA1c Measurement:

1. Tighten NGSP Manufacturer Certification Criteria
2. Tighten CAP Survey Grading for HbA1c
3. Reduce Interferences

Improving HbA1c Measurement:

1. Tightening of NGSP Manufacturer Certification Criteria:

- 1996: Started with initial criteria based on EP9 (bias) and EP5 (precision $\leq 5\%$); HbA1c range 4-14%
- 1999: Changed from EP9 bias assessment to Bland/Altman assessment of agreement (95% CI of differences within $\pm 1\%$ HbA1c)
- 2002: Tightened precision criteria from $\leq 5\%$ to $\leq 4\%$
- 2007 : tighten assessment of agreement criteria from $\pm 1\%$ to $\pm 0.85\%$ HbA1c (and narrowed the HbA1c range to 4-12%)
- 2010: Tightened assessment of agreement criteria from $\pm 0.85\%$ to $\pm 0.75\%$ HbA1c (and narrowed the HbA1c range to 4-10%).

Improving HbA1c Measurement

2. Changes in CAP Survey Grading for HbA1c

- 2007: Survey began accuracy based grading with $\pm 15\%$ acceptable limit
- 2008: Acceptable limit was reduced to $\pm 12\%$
- 2009: Acceptable limit was reduced to $\pm 10\%$
- 2010 Acceptable limit was reduced to $\pm 8\%$
- 2011-2012 Acceptable limit will be reduced to $\pm 7\%$

2010B CAP Pass Rates

Specimen	NGSP Target (% HbA1c)	Acceptable Range ($\pm 8\%$)	Pass Rate % (Low/High)	Cumulative Pass Rate %
GH2-04	5.2	4.8-5.6	81.4/100	95.3
GH2-05	8.7	8.0-9.4	71.4/100	95.1
GH2-06	6.3	5.8-6.8	89.4/100	97.1

CAP Pass Rates for 2010B using projected 2011 ($\pm 7\%$) cutoff

Specimen	NGSP Target (% HbA1c)	Acceptable Range ($\pm 7\%$)	Pass Rate % (Low/High)	Cumulative Pass Rate %
GH2-04	5.2	4.8-5.6	70.4/100	91.9
GH2-05	8.7	8.1-9.4	71.4/100	95.1
GH2-06	6.3	5.9-6.7	84.7/100	95.6

Improving HbA1c Measurement

3. Reducing Interferences

- Increasing awareness of HbA1c interferences
- Testing for interference from Hb variants for each method
- Encouraging use of methods without interference from Hb variants

Interferences from Hb Variants

- HbS is the most common hemoglobin variant in the US, followed by HbC and HbE.
- HbS is the most common variant worldwide followed by HbE and HbC.
- HbD (Punjab/Los Angeles) is the fourth most common variant in the US and worldwide

Method	Interference* from			
	HbAS	HbAC	HbAE	HbAD
Abbott Architect/Aeroset	Yes	Yes	-	-
Axis-Shield Afinion	No	No	No	No
Bayer A1cNOW	Yes	Yes	No	No
Beckman Synchron System	No	No	No	No
Bio-Rad D-10	No	No	No	No
Bio-Rad Variant II A1c	No	No	No	No
Bio-Rad Variant II Turbo A1c	No	No	Yes	Yes
Dade Dimension	No	No	No	No
Olympus AU system	Yes	Yes	No	No
Ortho-Clinical Vitros	No	No	No	No
Primus HPLC (affinity)	No	No	No	No
Roche Cobas Integra Gen.2	No	No	No	No
Roche/Hitachi (Tina Quant II)	No	No	No	No
Siemens Advia (original version)	Yes	Yes	-	-
Siemens DCA 2000	No	No	No	No
Tosoh A1c 2.2 Plus	No	No	Yes	No
Tosoh G7	No	No	Yes	No
Tosoh G8	No	No	Yes	No

*Clinical significance $\geq 10\%$ difference at 6 and 9% HbA1c

% of Labs Using Methods with Interference

(number of labs based on 2010 CAP survey data)

- 4.2% of laboratories are using methods with clinically significant* HbA**S** and /or HbA**C** interference.
- 21% of laboratories are using methods with clinically significant* HbA**E** interference#.
- 6.5% of laboratories are using methods with clinically significant* HbA**D** interference#.

*Clinical significance $\geq 10\%$ difference at 6 and 9% HbA1c

#Interference from HbD and E can usually be seen on HPLC chromatograms

Summary (1)

- Reporting of HbA1c is still not standardized globally but measurement units can be easily converted through linear equations. It appears that much of the world will eventually report HbA1c in IFCC mmol/mol.
- The US will continue to report NGSP %HbA1c and many laboratories also report an eAG.

Summary (2)

- HbA1c has been recommended for use in diabetes diagnosis.
- Accurate and precise measurement of HbA1c, especially near the normal range, is now even more important

Summary (3)

- HbA1c measurements have improved considerably since the DCCT ended in 1993.
- In an effort to further decrease the variability in HbA1c measurement, the NGSP will continue to tighten manufacturer certification criteria and the CAP will continue to tighten its PT criteria for laboratories.

Summary (4)

- Interference from Hb variants is still of concern but at the present time most laboratories are using methods that show no interference from the most common variants.
- The NGSP will continue to evaluate new methods for interference from the most common Hb variants.