Application of foreign assays on the BEP 2000.

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Capabilities of the BEP 2000.

Liquid handling

From reagents, 4 - 100 mL containers
From barcoded blood samples
To test tubes (1 tube per calibrator/sample)
To dilution plates (1 well per calibrator/sample)
To microtiter plate, assay plate
(multiple wells per calibrator/sample)

Incubation

At ambient + 5 °C to 50 °C
Tight temperature control
Carry over to neighbouring incubator

Measuring

Photometer within the BEP.
Any microplate reader outside the BEP. (flourometer, DELFIA meter)
Gamma counter for RIA tubes.

Challenges of the BEP 2000.

Which containers fit into the BEP?

4 mL and 20 mL vials from Zinsser Analytic GmbH, http://www.zinsser-analytic.com/
100 mL bottles from Dade-Behring.
1 liter wash bottles from Nalgene (catalogue no.: 2110-0032)

Challenges of the BEP 2000.

Communication with host computer (LABKA).

Since the BEP is a batch analyser, only one-way communication with host computer is needed.
A *.txt file can be generated for each run, and data can be transferred to host.

Challenges of the BEP 2000.

Assay evaluation.

Optimize reagent concentrations at 37 °C.

Optimize incubation times at 37 °C.

Optimize colour development at 32 or 37 °C.

Expected shape of calibration curve.

Calibration curve within 0 to 2.5 OD-units.

With-in run variation / plate homogeneity.

Between run variation.

Dilution of high / positive samples.

Comparison with “old” method / “reference” method, using more than 100 samples.

Reference interval: 120 - 150 blood donors.

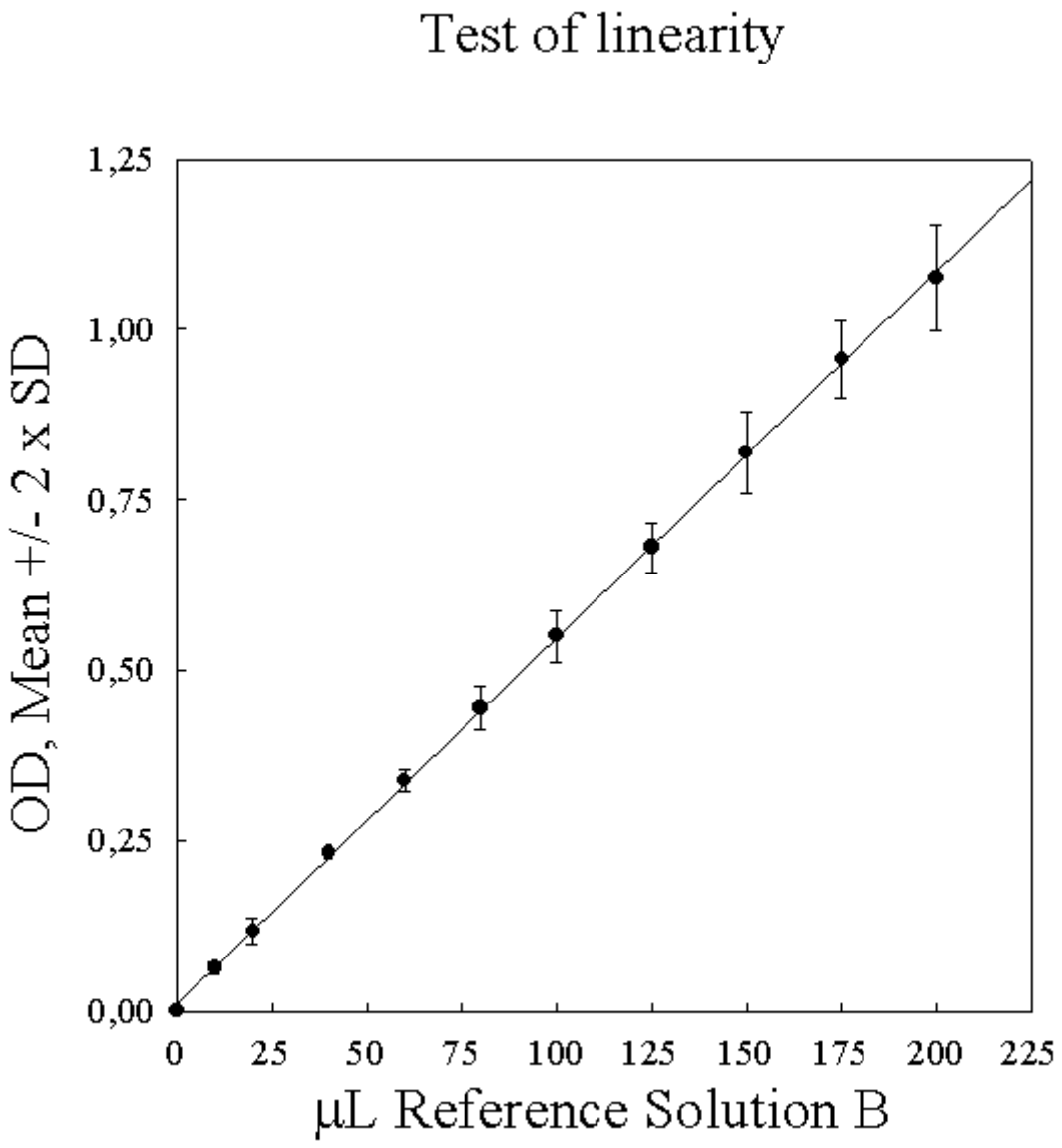
Assay of samples from external control schemes.

Challenges of the BEP 2000.

Test of linearity with Reference Solution B.

Volume	OD	CV, %
4 x (2 x 0,010 mL)	0,063	6,9
4 x (2 x 0,020 mL)	0,117	7,7
4 x (2 x 0,040 mL)	0,232	1,9
4 x (2 x 0,060 mL)	0,338	2,4
4 x (2 x 0,080 mL)	0,445	3,5
4 x (2 x 0,100 mL)	0,550	3,5
4 x (2 x 0,125 mL)	0,680	2,6
4 x (2 x 0,150 mL)	0,818	3,7
4 x (2 x 0,175 mL)	0,956	3,0
4 x (2 x 0,200 mL)	1,075	3,6

Volume adjusted to 0,225 mL with detergent containing buffer (wash buffer).


Test of linearity
OD, Mean +/- 2 x SD
1,25
1,00
0,75
0,50
0,25
0,00
0
25
50
75
100
125
150
175
200
225
µL Reference Solution B

Challenges of the BEP 2000.

Test of precision with Reference Solution B.

Volume	OD	CV, %
4 x (2 x 0,025 mL)	0,144	6,0
8 x (1 x 0,025 mL)	0,139	3,5
8 x (1 x 0,200 + 0,025 mL)	0,139	0,9
8 x (1 x 0,300 + 0,025 mL)	0,129	1,2
4 x (2 x 0,025 mL)	0,143	6,6
8 x (1 x 0,025 mL)	0,141	2,9
8 x (1 x 0,200 + 0,025 mL)	0,140	0,6
8 x (1 x 0,300 + 0,025 mL)	0,131	0,8
8 x (1 x 0,100 mL)	0,528	0,7
8 x 0,100 mL	0,546	2,1

Volume adjusted to 0,225 mL with detergent containing buffer (wash buffer) for wells with only 0,025 mL reagent.

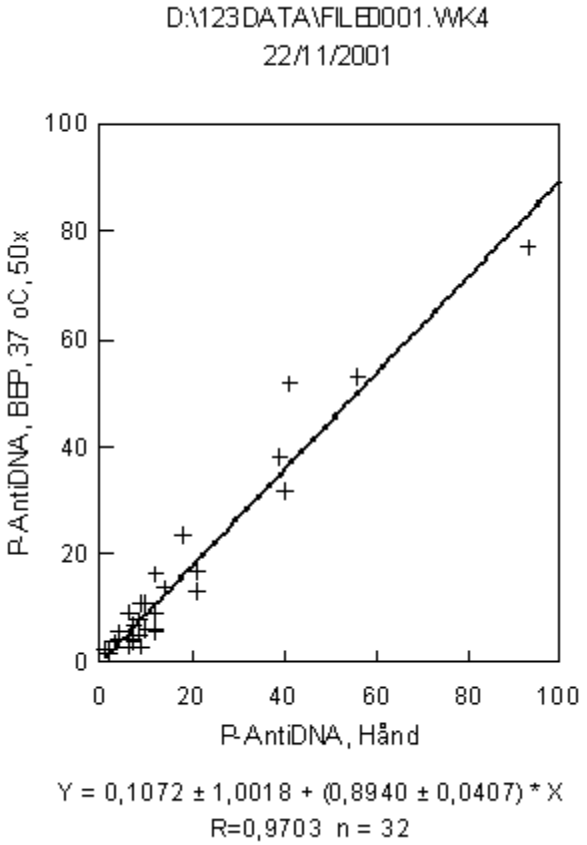
Challenges of the BEP 2000.

Most kits are developed for 25 °C

Case 1: Calibrators and samples are different.

Problem: The results from P-Anti-DNA analysis at 37 °C are 50 % of the results at ambient winter temperature.

Solution: We use 50-fold pre-dilutions in stead of 100-fold pre-dilutions.

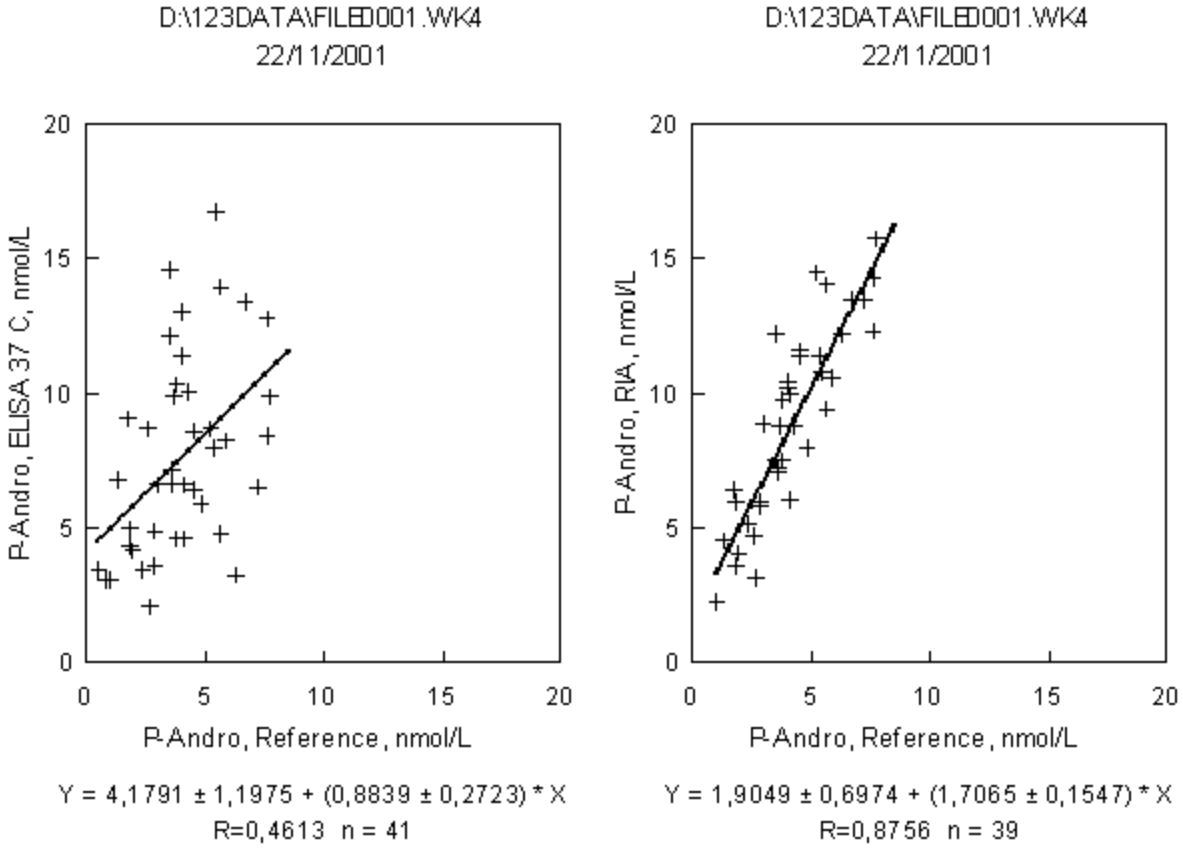


??????: Is the clinical sensitivity and clinical specificity unchanged?

Challenges of the BEP 2000.

Is the chemical specificity indifferent to temperature?

Case 2: P-Androstendion.



Solution: Change of method to radio-immuno assay.

Current use of BEP2000.

P-DNA antibodies

P-Sex hormone binding globulin (P-SHBG)

P-Dehydro epi androstenedion sulphate (P-DHEAS).

This document is posted on the internet at the address:
http://www.auh.dk/akh/afd/klinbio/dk/ansat/fen/bep_2001.pdf

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