Establishing reference values

- with low number of controls -

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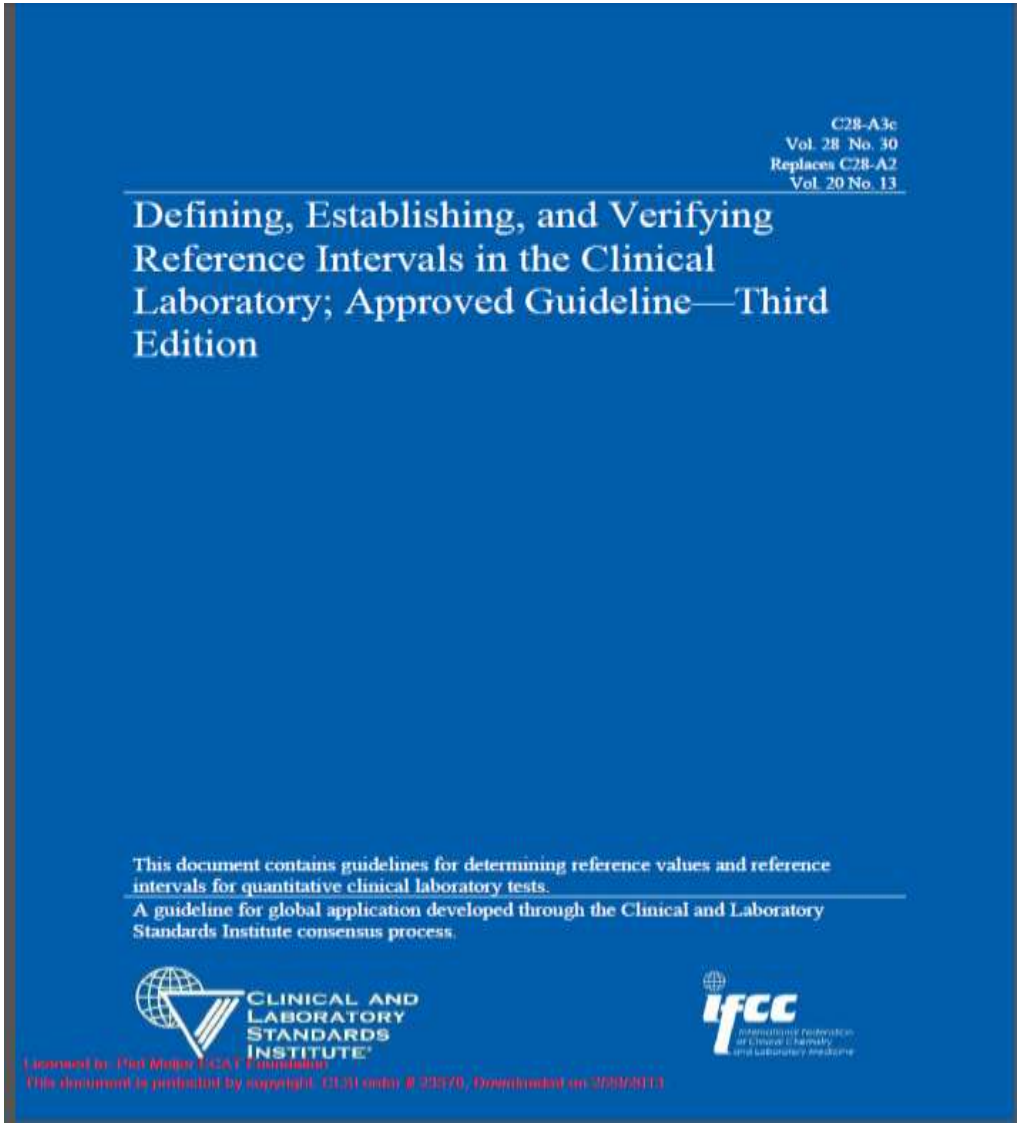
CLSI EP28-A3C



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- Establish a reference interval:
  - Use of nonparametric method if the laboratory has limited access to statistical and computational support.
  - Minimum sample size: 120 control samples for 90% CI
    - Distribution independent
    - CI nonparametrically
    - (width 90% CI/width 95% RI) <0.2

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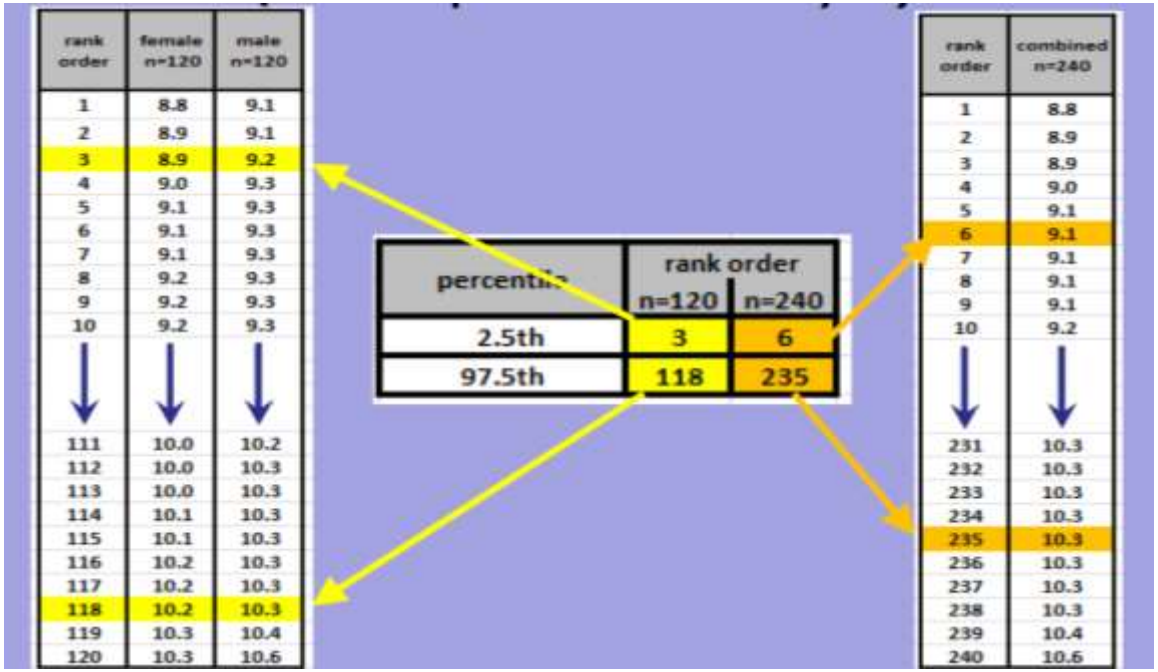
Nonparametric method

90% CI lower

90% CI upper



rank order	female n=120	male n=120
1	8.8	9.1
2	8.9	9.1
3	8.9	9.2
4	9.0	9.3
5	9.1	9.3
6	9.1	9.3
7	9.1	9.3
8	9.2	9.3
9	9.2	9.3
10	9.2	9.3
111	10.0	10.2
112	10.0	10.3
113	10.0	10.3
114	10.1	10.3
115	10.1	10.3
116	10.2	10.3
117	10.2	10.3
118	10.2	10.3
119	10.3	10.4
120	10.3	10.6

percentile	rank order n=120	rank order n=240
2.5th	3	6
97.5th	118	235

rank order	combined n=240
1	8.8
2	8.9
3	8.9
4	9.0
5	9.1
6	9.1
7	9.1
8	9.1
9	9.1
10	9.2
231	10.3
232	10.3
233	10.3
234	10.3
235	10.3
236	10.3
237	10.3
238	10.3
239	10.4
240	10.6

Horowitz, ECAT 2012

90% CI upper: (N+1) – rank number

Sample size	Rank numbers	
	Lower	Upper
119–132	1	7
133–160	1	8
161–187	1	9
188–189	2	9
190–218	2	10
219–248	2	11

What if less than 120 samples?

- Verify reference interval:
  - Method comparison
  - Verification experiment

- Establish reference interval:
  - Parametric or robust method

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Verifying reference interval

a) **Method comparison**

- At least 40 random samples which cover substantial portion of reportable range.
- All measurements in duplicate.
- Methods statistically identical with 95% confidence if: 1 in CI slope, 0 in CI intercept.

b) **Verification experiment**

- At least 20 healthy control individuals.
- > 90% within defined reference interval, data passes verification and the stated reference interval is validated.

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What if less than 120 samples?

- Verify reference interval:
  - Method comparison
  - Verification experiment

- Establish reference interval:
  - Parametric or robust method

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CLSI recommendation

Among these three latter methods, the working group would like to call attention to the "robust method," because it may offer the best way of dealing with limited numbers of observations. The robust method can be thought of as a compromise between the parametric and nonparametric methods. It has the appeal of the parametric method in that it does not require as many observations as the nonparametric procedure, and yet it does not require that the underlying population of analytical values follow a Gaussian distribution. This method has the same form as the parametric except, instead of the mean and standard deviation of the sample, robust measures of location and spread are used. The robust method has been used in a variety of situations where the available sample size is less than 120, but where the underlying population is not assumed to follow a Gaussian distribution. Details on the computations involved may be found in Horn and Pesce, 2005.[36]

Robust method:

- Less samples than nonparametric method (like for parametric method)
- No Gaussian distribution required

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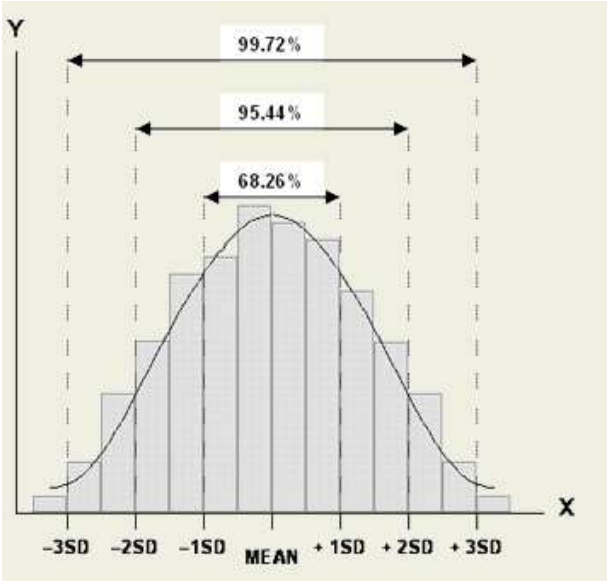
Parametric method

- Gaussian distribution required (after transformation)
- Mean ± 2SD
- (width 90% CI/width 95% RI) <0.2



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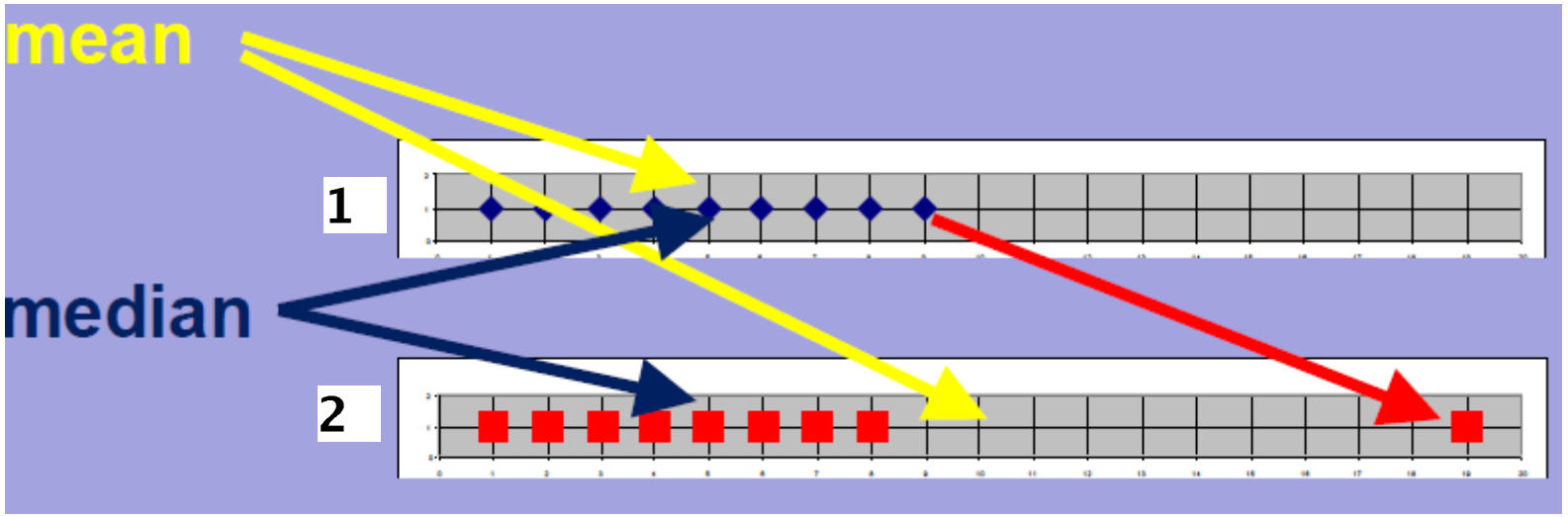
Robust method

- Build on median obtained by an iterative process.
- Method is less sensitive to data further away from the central location.



Horowitz, ECAT 2012

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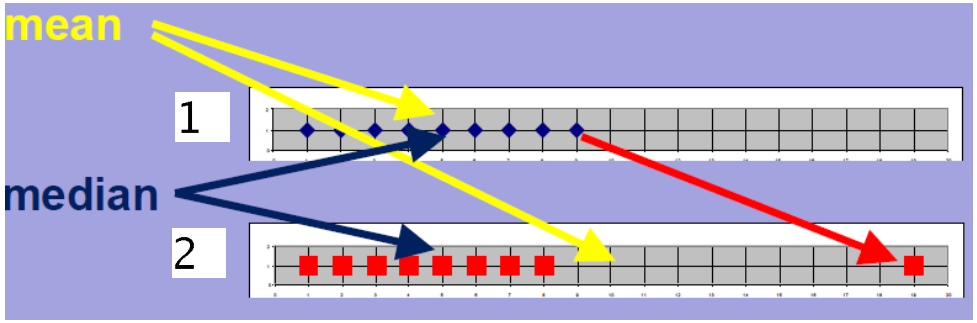
Robust method

- Gaussian distribution is not required
- Symmetry of data required (after transformation)?
- (width 90% CI/width 95% RI) <0.2



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Study

- Usability different statistical methods for the calculation of reference intervals for coagulation tests:
  - Different statistical software programs
  - 20-120 control samples

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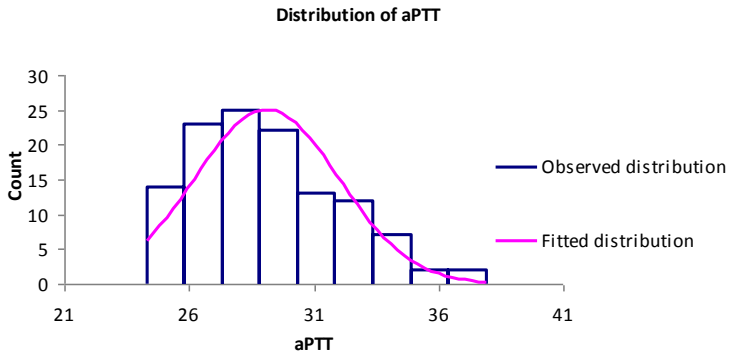
Coagulation (screening)tests

– nonparametric versus parametric/robust –

- 120 control patients (adults)
- APTT (Cephascreen), PT (Neoplastin R) and fibrinogen measured on STARev
- Statistical software programs:
  - Reference Value Advisor
  - EP evaluator
- No Gaussian distribution:



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Coagulation (screening)tests

– nonparametric versus parametric/robust –

N=120	APTT (sec)	PT (sec)	Fibrinogen (g/l)
nonparametric Reference value advisor/ EP– evaluator	24.8–36.1	12.1–16.5	2.4–5.7
parametric			
– EP evaluator	24.0–35.1	11.9–15.5	2.3–5.5
– Reference value advisor	24.5–36.1	12.2–16.0	2.3–5.6
robust/untransf.	23.1–34.6	11.5–15.4*	1.8–5.2*
robust/Box–Cox	24.5–36.0	12.2–16.0*	2.3–5.6

* No symmetry of data

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Nonparametric versus parametric

N=120	APTT (sec)	PT (sec)	Fibrinogen (g/l)
Nonparametric	24.8–36.1	12.1–16.5	2.4–5.7
90% CI lower	24.3–25.4	12.1–12.4	2.2–2.5
90% CI upper	34.7–37.9	15.0–18.2	5.2–5.9 0.21
	0.28	0.73	
Parametric, RvA	24.5–36.1	12.2–16.0	2.3–5.6
90% CI lower	24.1–24.9	12.1–12.4	2.2–2.4
90% CI upper	34.8–37.5	15.5–16.6	5.2–6.0 0.24
	0.23	0.29	
Parametric, EP	24.0–35.1	11.9–15.5	2.3–5.5
90% CI lower	23.4–24.6	(width 90% CI/width 95% RI) 4	

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Nonparametric versus Robust

N=120	APTT (sec)	PT (sec)	Fibrinogen (g/l)
Nonparametric	24.8–36.1	12.1–16.5	2.4–5.7
90% CI lower	24.3–25.4	12.1–12.4	2.2–2.5
90% CI upper	34.7–37.9	15.0–18.2	5.2–5.9
	0.28	0.73	0.21
Robust *untransf*	23.1–34.6	11.5–15.4*	1.8–5.2*
90% CI lower	22.2–23.7	11.1–11.9	1.6–2.0
90% CI upper	33.7–35.5	15.0–15.8	4.9–5.4
	0.16	0.21	0.15
Robust *Box–Cox*	24.5–36.0	12.2–16.0*	2.3–5.6
	24.0–24.9	12.1–12.4	2.2–2.4

* No symmetry of data

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Less than 120 samples

APTT	nonparametric	parametric EP evaluator	robust untransf Reference value Advisor
N=120 non-G	24.3–36.1	24.0–35.1	23.1–34.6
CI upper limit	**34.7**–37.9 0.28	**34.2**–35.9 0.15	**33.7**–35.5 0.16
N=80 Gaussian	24.3–37.9	24.4–36.4	23.4–35.9
CI upper limit	**35.2**–38.3 0.23	**35.2**–37.6 0.20	**34.8**–37.1 0.18
N=40 Gaussian	24.3–39.6	24.3–37.3	23.7–37.3
CI upper limit	**36.1**–39.6 0.23	**35.8**–38.8 0.23	**35.8**–39.2 0.25
N=20 Gaussian	-	26.2–38.8	25.3–39.2
CI upper limit		36.7–40.8 0.33	36.6–41.5 0.35

PT	nonparametric	parametric EP evaluator	robust untrans Reference value Advisor
N=120 non-G	12.1–16.5	11.7–15.5	11.5–15.4*
CI upper limit	15.0–18.2 0.73	15.2–15.7 0.13	15.0–15.8 0.21
N=80 non-G	12.2–17.1	11.9–15.8	11.4–15.7*
CI upper limit	15.3–18.2 0.59	15.4–16.1 0.18	15.1–16.2 0.26
N=40 Gaussian	12.1–17.1	12.0–15.9	11.7–15.9
CI upper limit	15.4–17.1 0.34	15.4–16.3 0.23	15.2–16.5 0.31
N=20 Gaussian	-	12.1–16.4	11.7–16.4
CI upper limit		15.7–17.1 0.33	15.4–17.2 0.38

* No symmetry of data

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Conclusions

- Rijnstate population of control samples:
  - Nonparametric method:
    - CI/RI decreases with lower numbers of controls: influence population distribution??
  - Parametric method:
    - Different results for EP evaluator and Reference Value Advisor.
  - Robust method:
    - Results are closest to parametric method with EP evaluator, independent of population distribution.

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Conclusions

- Selection of the statistical method can have influence on the width of the 90% CI.
- The same statistical method with various software programs can result in different outcomes!
- Rijnstate control population:
  - Robust method had no advantages over parametric method with low number of control samples.
  - Lower limit of upper CI (of the RI): similar results for nonparametric, parametric, robust method (independent of number of control samples).

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