

# Investigation of Cleaning Efficiency conducted by Cleaning Evaluation/Determination Working Group in Japanese Hospital Settings



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# Target of Investigation

- Establishment of acceptance criteria of residual protein on a reprocessed instrument

Background:

- Growing awareness of cleaning efficiency
- Wide-spread use of WDs
- Popularized residual protein detection

# Hierarchic Structure of Working Group

- JAPANESE SOCIETY OF MEDICAL INSTRUMENTATION (JSMI)

- Certification Committee of Reprocessing Technicians

- Cleaning Evaluation/Determination Working Group

WG was set up in April 2006 for cleanliness evaluation with 11 group members assigned. The investigation was conducted in 7 hospitals.

# Members of Working Group

<b>Ryo Fushimi</b>	Osaka Univ. Hosp.
<b>Yushi Uetera</b>	Univ. of Tokyo Hosp.
<b>Kazuhisa Matsuda</b>	Saiseikai Fukuoka General Hosp.
<b>Yutaka Shimazaki</b>	Kainan Hosp. Aichi Prefectural Welfare Federation of Agricultural Cooperatives
<b>Toshiaki Matsuyama</b>	Univ. Hosp. of Occupational & Enivironmental Health
<b>Ken Sakai</b>	Kyushu Univ. Hosp.
<b>Hisami Tanimura</b>	Kanto Medical Center NTT EC
<b>Yoji Harada</b>	Clean Chemical Co., Ltd.
<b>Masatake Shimizu</b>	Inui Medics Corporation
<b>Hiroyoshi Kobayashi</b>	Tokyo Healthcare Univ.
<b>Tomozo Yamamoto</b>	Muranaka Medical Instruments Co., Ltd.

# Utilized WDs in Hospital Surveyed

**WD:** single-chamber, **US:** ultrasonic bath

**TWD:** multi-chamber WD, **TUS:** multi-chamber ultrasonic bath

**w/US:** built-in, **+US:** separate installation

WD	Miele <sup>□</sup>	G7736	
WD	Hamo <sup>□</sup>	T-21	
WD w/US	Udono <sup>□</sup>	UKW	
WD+US	Deko <sup>□</sup>	2000E	Sakura <sup>□</sup> US-202A
TUS	Sakura <sup>□</sup>	US-87517	
TWD w/US	Hamo <sup>□</sup>	T-840	
TWD w/US	Hamo <sup>□</sup>	T-940	

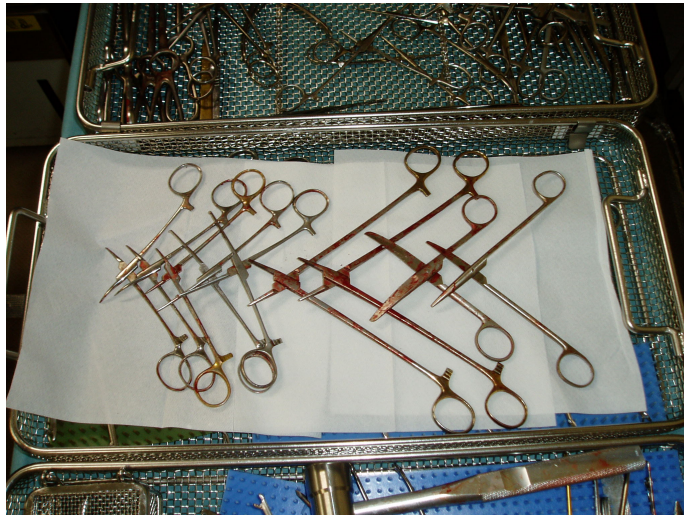
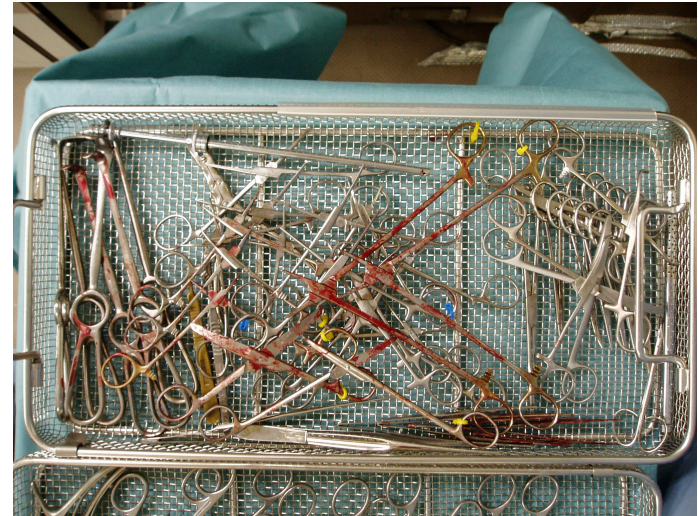
# Cleaning Programs

t emp °C/min., A: alkaline detergent, E: enzymatic detergent

Hos p.	type	pre- rinse	main wash	interim rinse	ultrasonic wash	interim rinse	interim rinse	final rinse
A	WD	-/5	60°C/5 A	---	---	---	---	93°C/10
B	WD	-/5	93°C/10 A	---	---	-/1	-/1	75°C/1
C	WD w/US	40°C/10	40°C/15 E	40°C/3	40°C/10 E	40°C/3	40°C/3	93°C/10
D	WD (1st) + US (2nd)	-/3	50°C/5 A	---	---	-/5	---	93°C/10
		---	---	---	-/8 A	-2	-/2	-/2
	TUS	50°C/10	---	---	50°C/10 A	---	50°C/7	93°C/3
E	TWD	35°C/4.5	40°C/12 E	---	40°C/12 E	-/2.25	-/3.25	93°C/11.25
F	TWD	-/2	60°C/10 A	-/1	-/10 none	-/1	-/1	93°C/10
G	TWD	-/5	40°C/5 E	---	55°C/5 E	-/1	-/1	90°C/5

# Clinical Test pieces

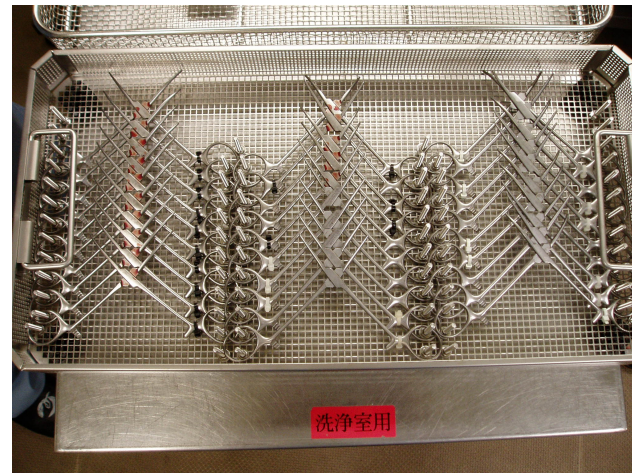
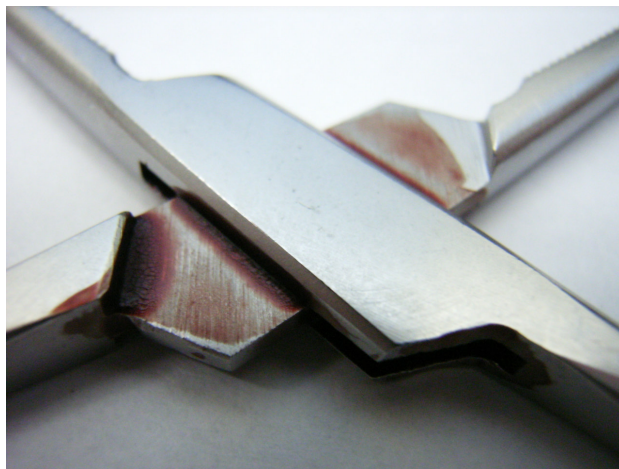
- Needle Holders
- Hemostatic Forceps
- Scissors





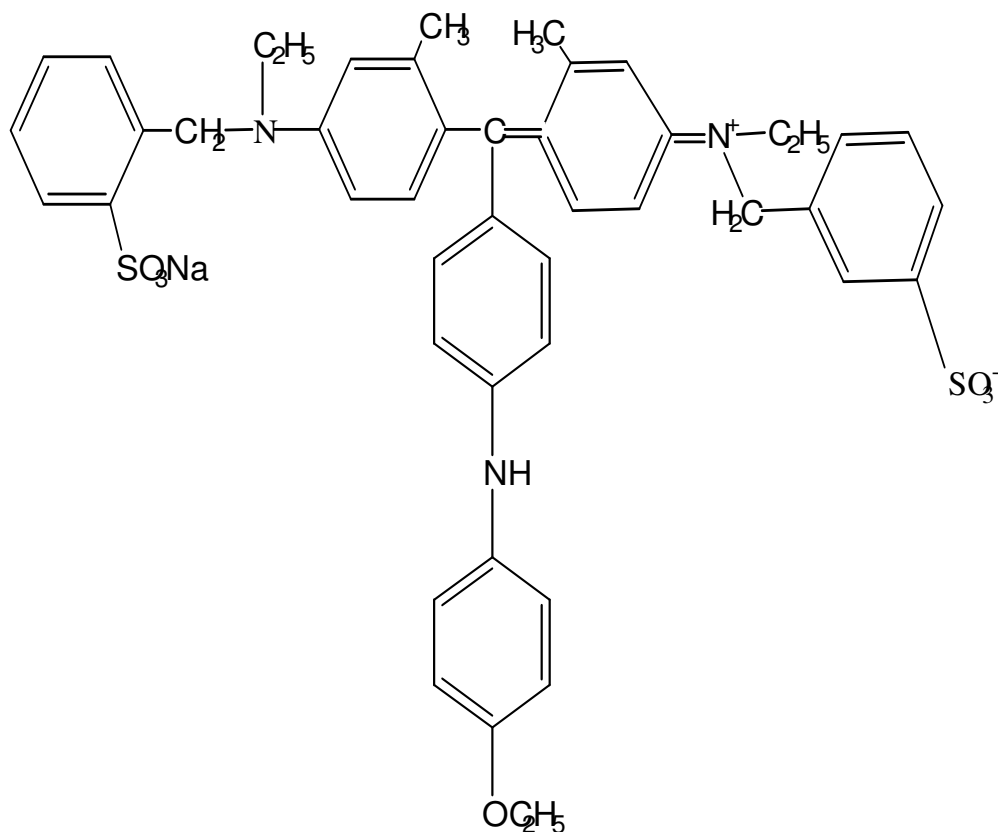
# Artificial Test Pieces

- Inoculate 100  $\mu$ L of Heparinized Sheep Blood with Protamine Sulfate on a forceps
  - Stored 24hrs. after inoculation at 25°C, 75%RH in a tray under the defined configuration
  - Sealed in polyethylene (PE) bag and delivered to 7 hospitals after overnight storage
  - Cleaned by WDs on 4 days after inoculation

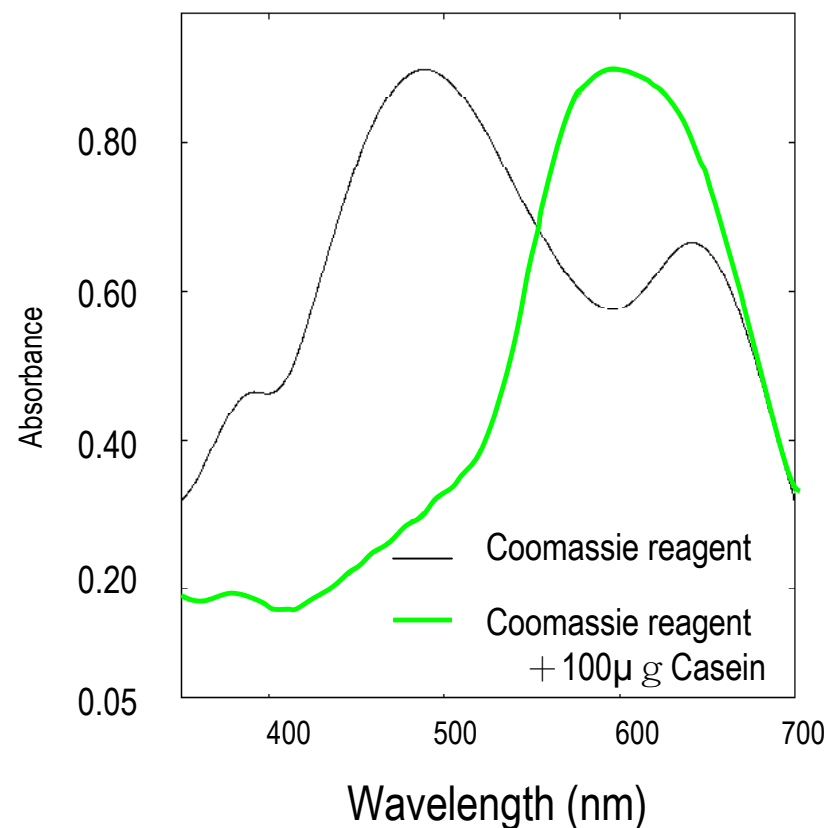




# Structural Formula and Absorption Spectrum of Coomassie Brilliant Blue G-250



Structural formula

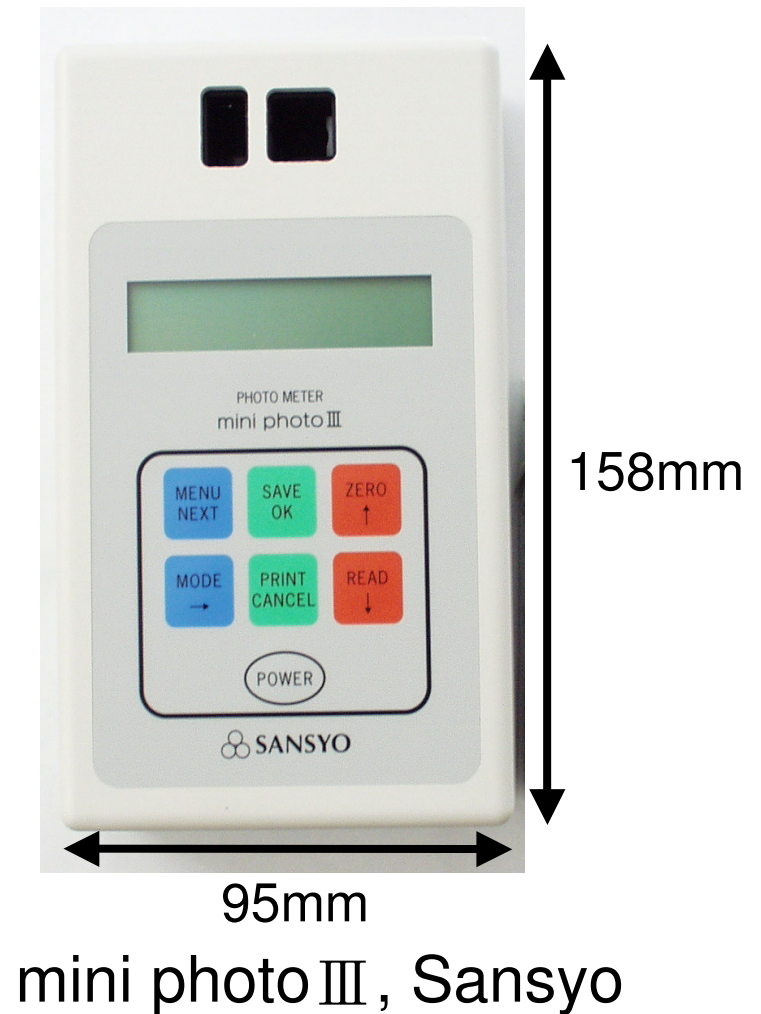
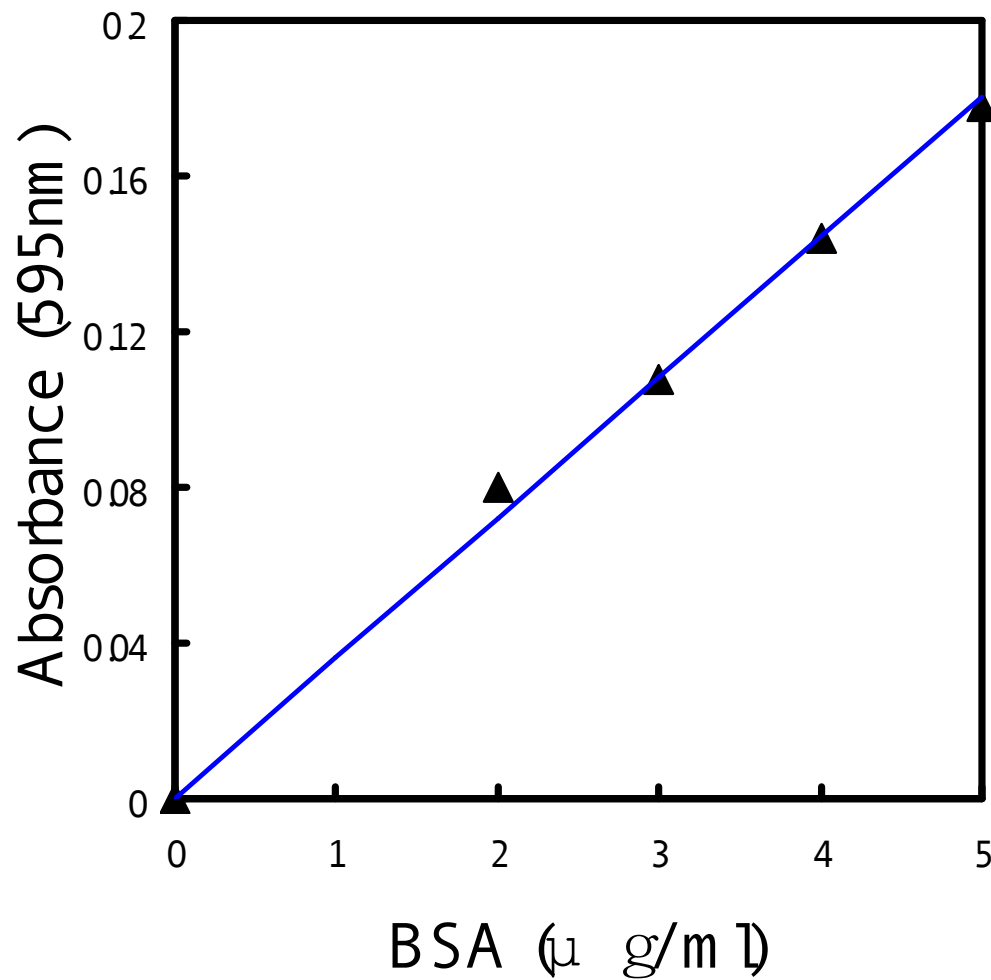


Absorption spectrum

# Sensitivity and Accuracy of Protein Assay with Coomassie Brilliant Blue G-250

BSA applied ( $\mu\text{g}$ )	5	10	20	30	40	50
BSA ( $\mu\text{g}$ , N= 5 )	<b>7.7</b>	<b>9.1</b>	<b>19</b>	<b>28.7</b>	<b>39.1</b>	<b>47.2</b>
	<b>4.3</b>	<b>9.4</b>	<b>21.3</b>	<b>30.9</b>	<b>38.2</b>	<b>48.5</b>
	<b>7.3</b>	<b>10.2</b>	<b>21.5</b>	<b>30.7</b>	<b>41.8</b>	<b>49.8</b>
	<b>6.3</b>	<b>10.8</b>	<b>19.5</b>	<b>30.1</b>	<b>42.1</b>	<b>47.3</b>
	<b>2.9</b>	<b>8</b>	<b>19.6</b>	<b>32.9</b>	<b>41.7</b>	<b>51.2</b>
Average ( $\mu\text{g}$ )	<b>5.7</b>	<b>9.5</b>	<b>20.2</b>	<b>30.7</b>	<b>40.6</b>	<b>48.8</b>
Standard Deviation	<b>2.0</b>	<b>1.1</b>	<b>1.1</b>	<b>1.5</b>	<b>1.8</b>	<b>1.7</b>
Coefficient of Variation	<b>0.36</b>	<b>0.11</b>	<b>0.06</b>	<b>0.05</b>	<b>0.04</b>	<b>0.03</b>

# Calibration Curve of CBB Method and Portable Photometer



# CBB Procedure for Clinical Test Pieces

1. Put a test piece into a polyethylene (PE) bag containing 10 ml of 0.2 mol/l sodium hydroxide extract.
2. Place the test piece packed in PE bag into an ultrasonic bath to extract residual protein at 50°C for 30 min.
3. React 1 ml of the extract to 3 ml of the Coomassie reagent at 25°C for 20 min.
4. Determine the absorbance of the reactant by spectrophotometry at 595nm, and calculate the overall quantity of residual protein on the test piece according to the calibration curve.

# CBB Procedure for Artificial Test Pieces

Additional steps mentioned below were inserted between the step 2nd and 3rd in the CBB procedure for clinical test pieces to improve the recovery rate.

1. Decant 2ml of the extract into a small PE pouch from a PE bag right after ultrasonic processing.
2. Open/close a forceps 200 times for approx. 2min.
3. Decant 2ml of the extract from PE pouch back again into the original PE bag.

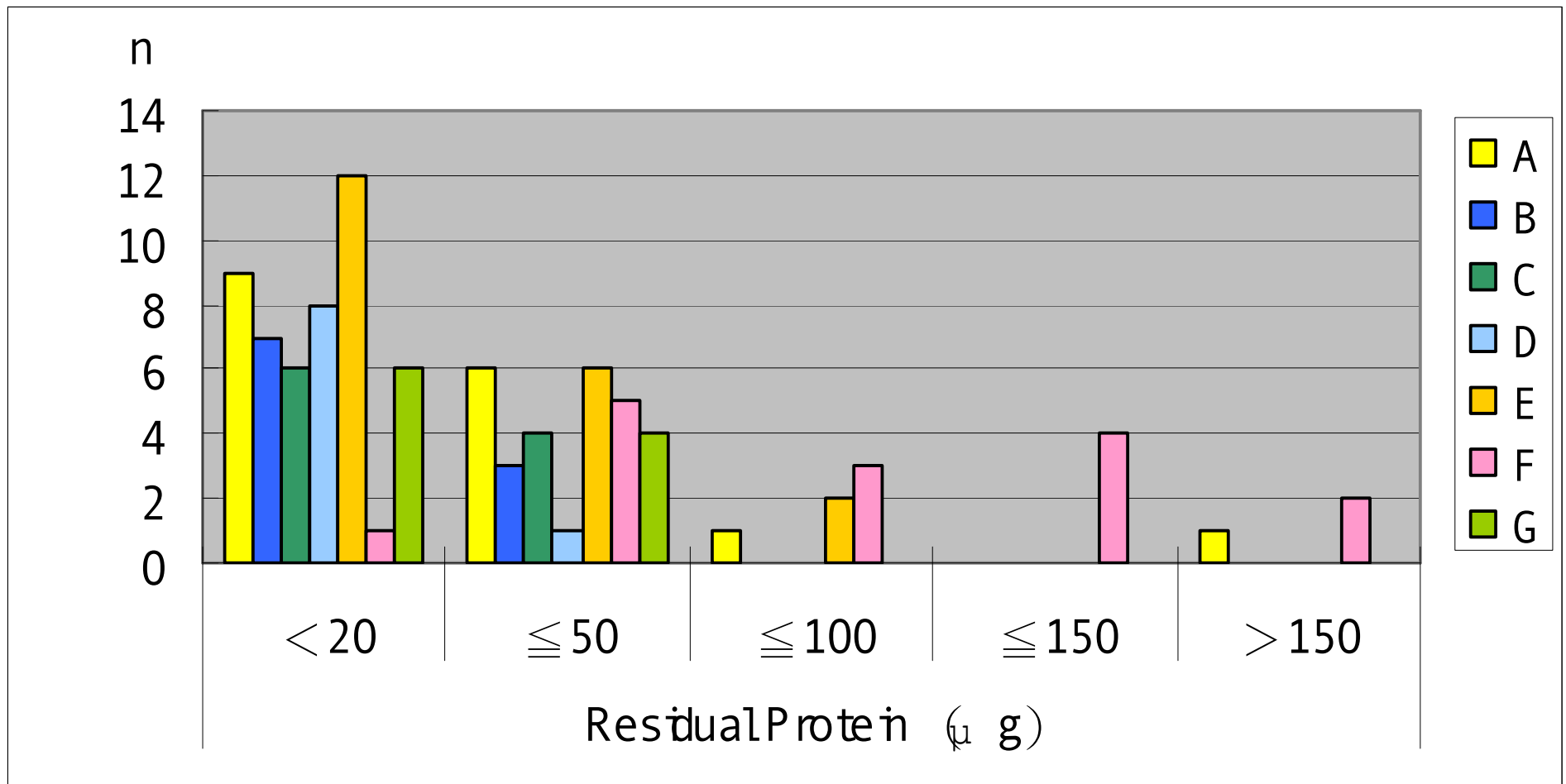


# Cleaning Result of Clinical Test Pieces determined by CBB Method

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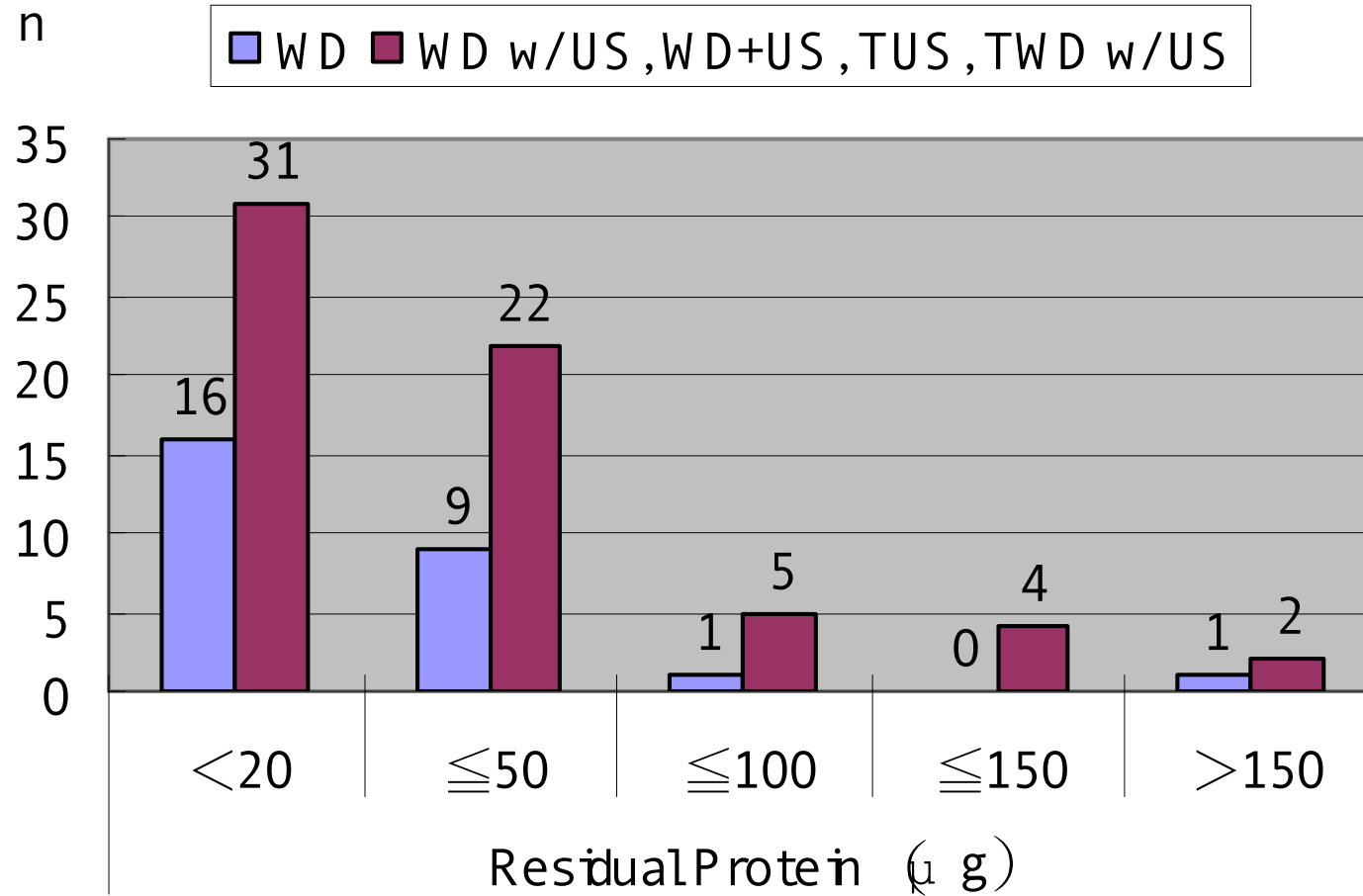


# Cleaning Result of Clinical Test Pieces determined by CBB Method (independent result of 7 hospitals)

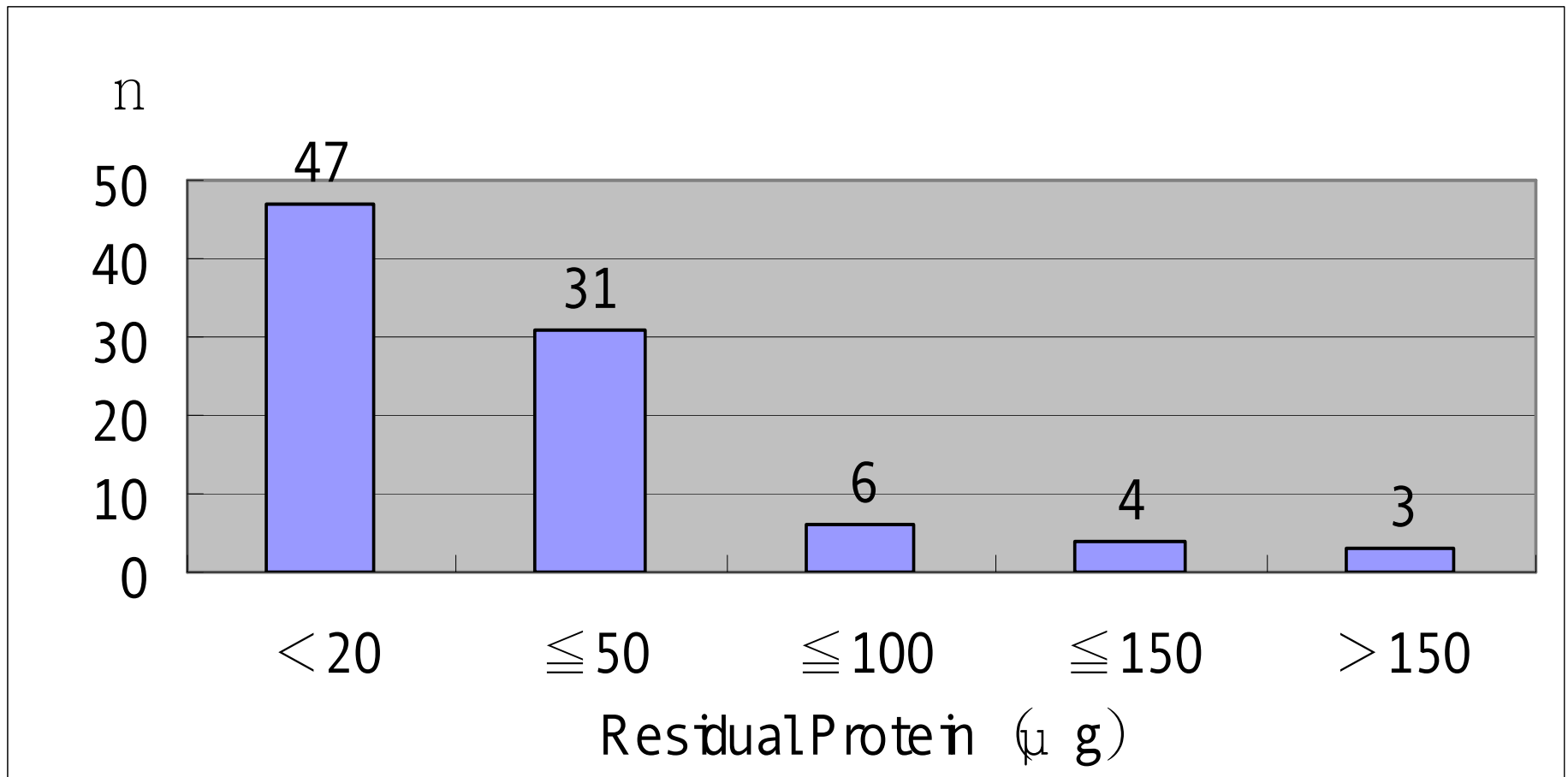


# Cleaning Result of Clinical Test Pieces

## Ultrasonic Effect



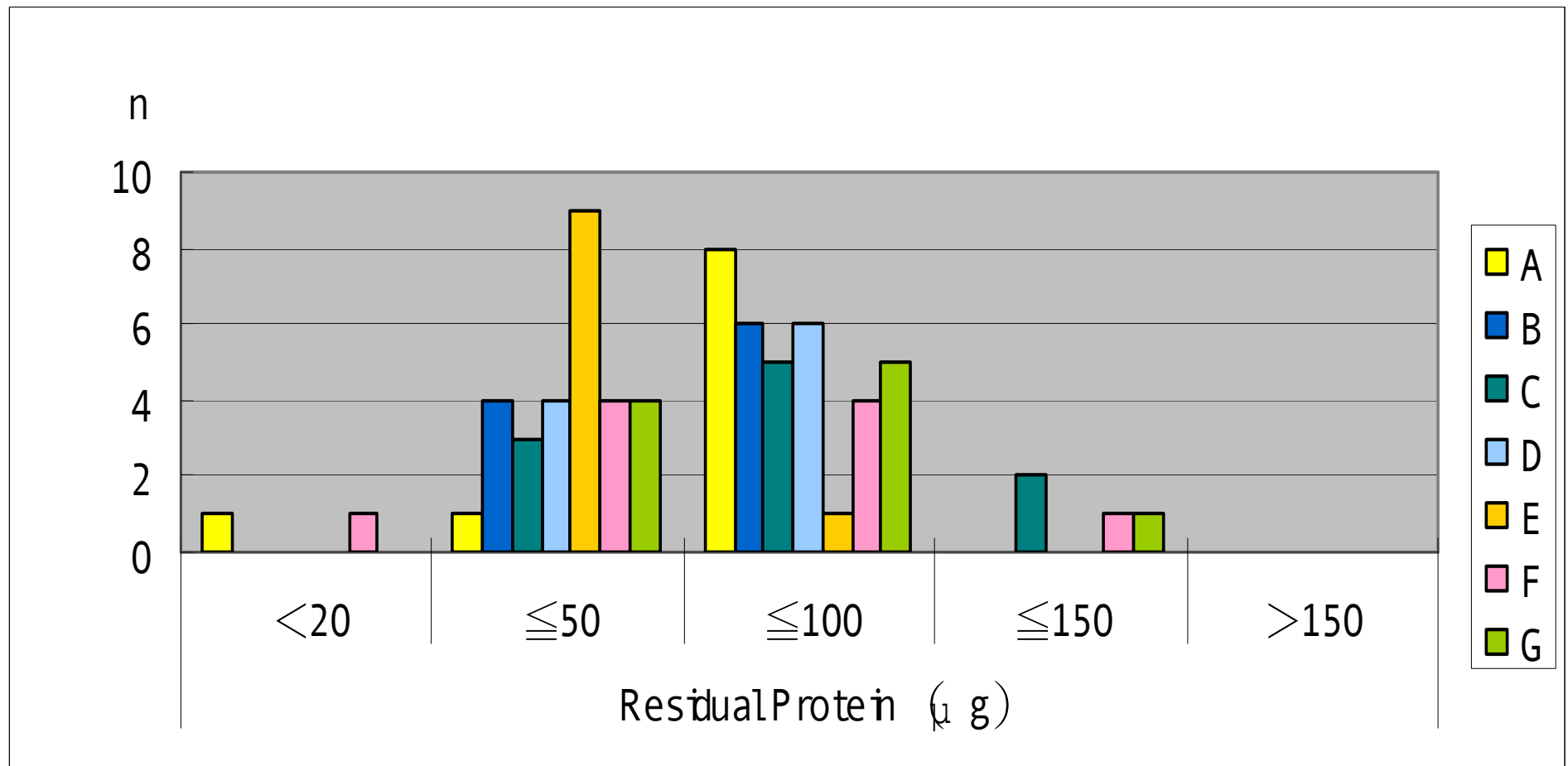
# Cleaning Result of Clinical Test Pieces determined by CBB Method



# Cleaning Result of Artificial Test Pieces contaminated by Sheep Blood determined by CBB Method

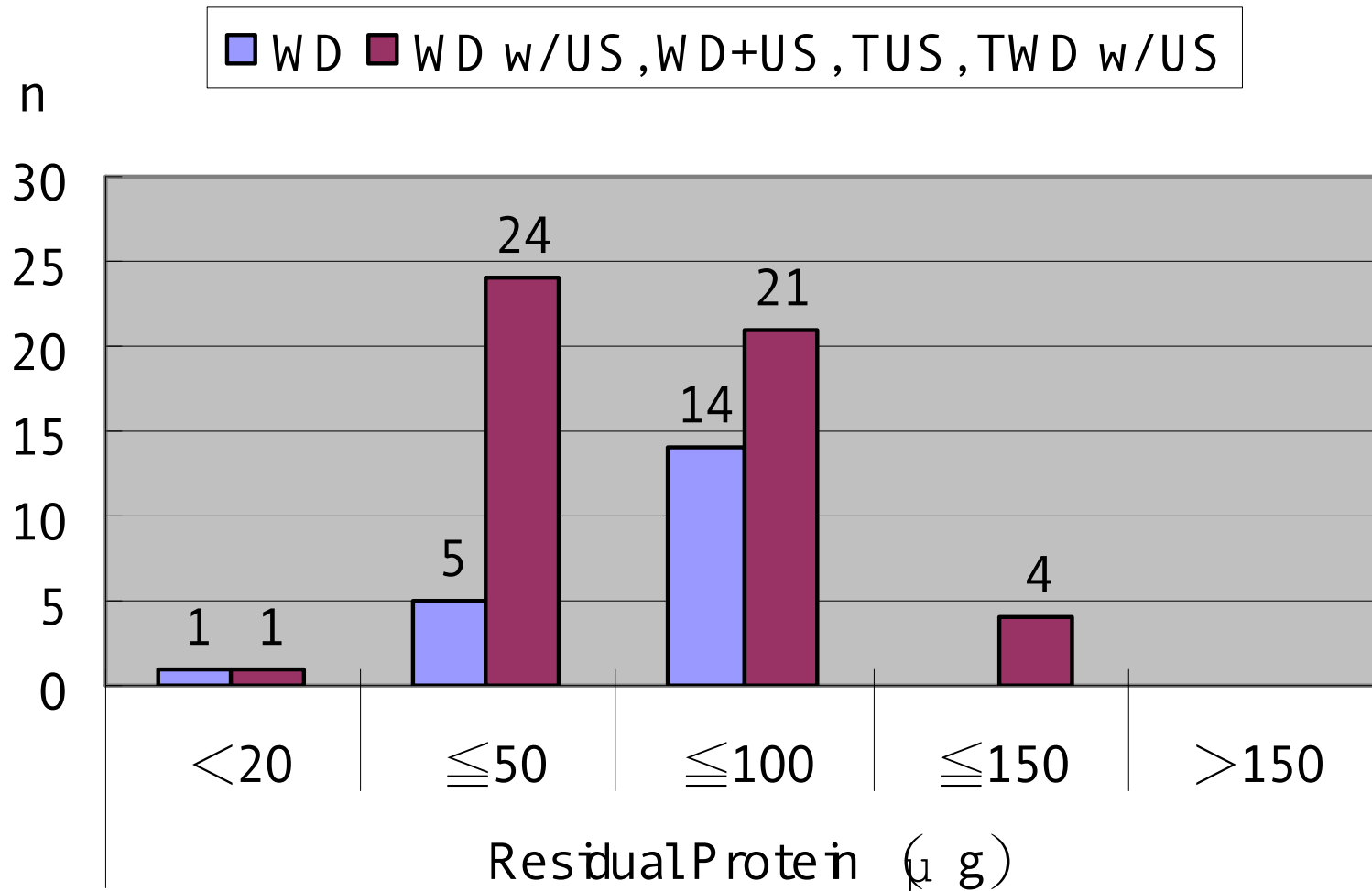
Hos p.	Residual Protein (μg)									
	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8	No.9	No.10
<b>A</b>	<20	30	51	60	62	65	75	75	79	84
<b>B</b>	31	33	40	41	55	60	65	68	74	90
<b>C</b>	27	39	47	76	80	86	91	99	101	114
<b>D</b>	31	33	40	41	55	60	65	68	74	87
<b>E</b>	24	29	32	33	34	34	35	41	46	55
<b>F</b>	<20	22	26	28	39	57	62	77	87	101
<b>G</b>	28	34	39	42	60	60	86	96	100	104

# Cleaning Result of Artificial Test Pieces determined by CBB Method (independent result of 7 hospitals)



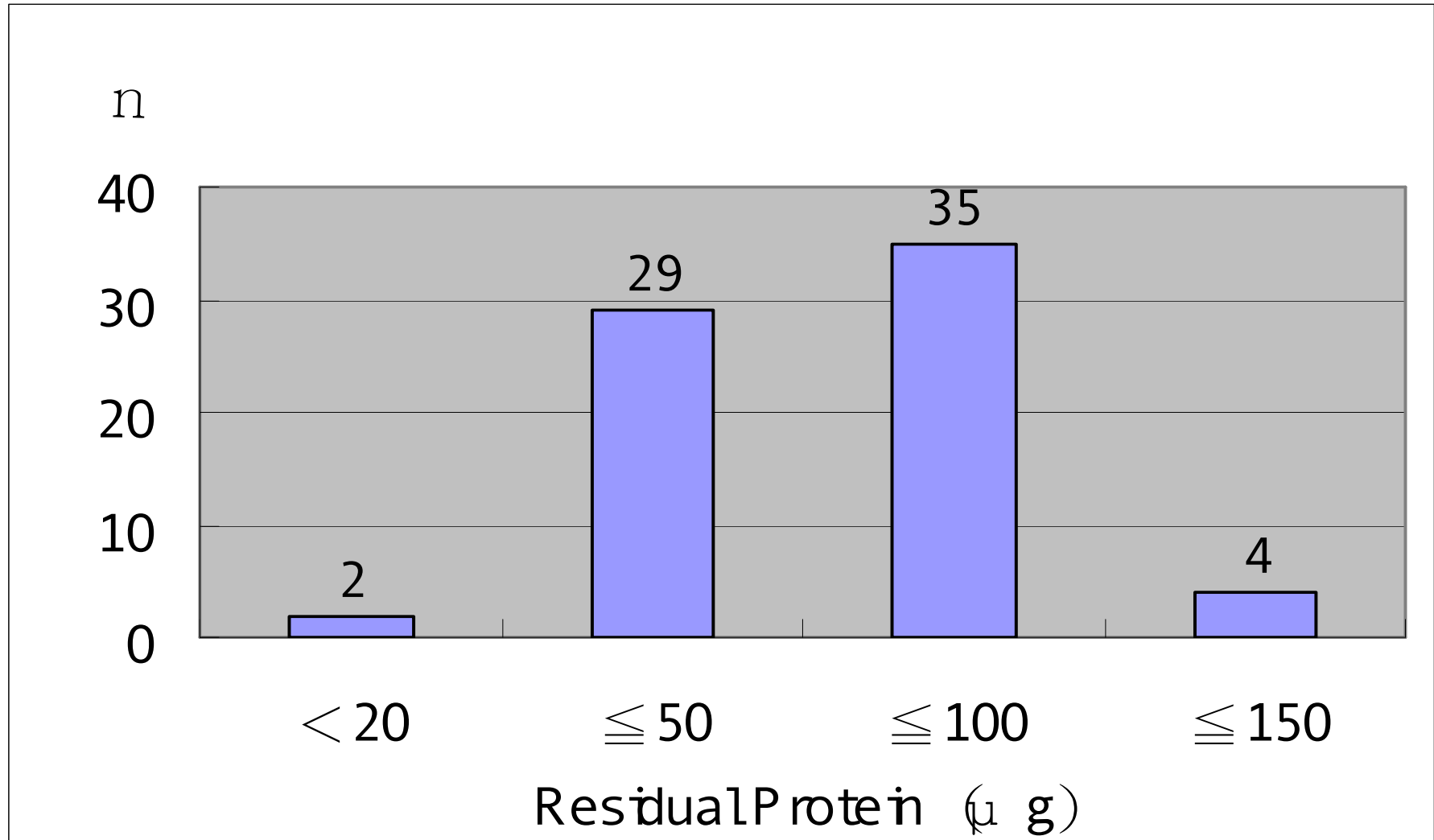
# Cleaning Result of Artificial Test Pieces

## Ultrasonic Effect





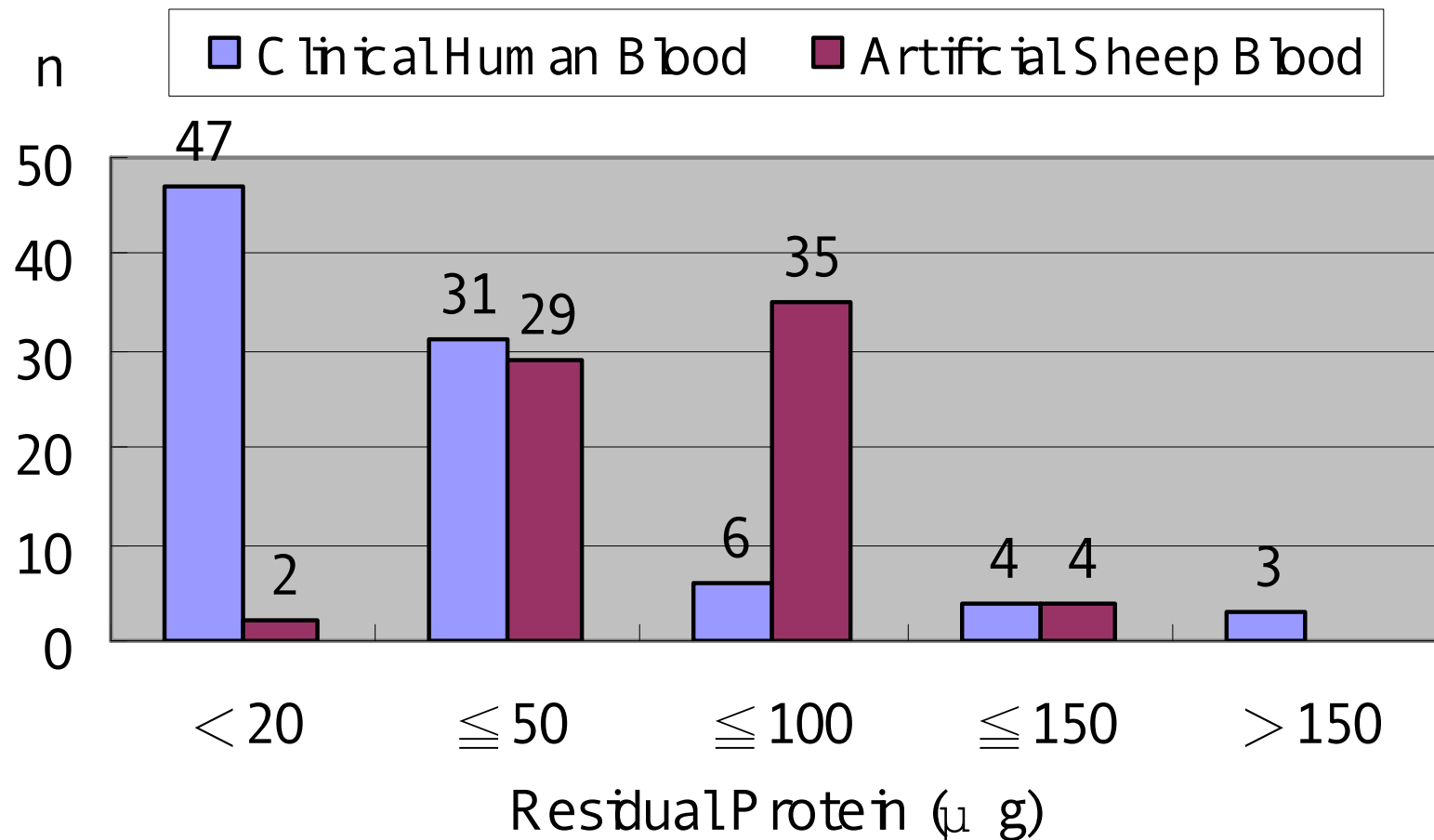
# Cleaning Result of Artificial Test Pieces contaminated by Sheep Blood determined by CBB Method



# Overall Cleaning Result of Clinical & Artificial Test Pieces

	<b>Average</b> ( $\mu\text{g}$ )	<b>Standard Deviation</b> ( $\mu\text{g}$ )	<b>&lt; 20</b> (pcs.)	<b><math>\leq 50</math></b> (pcs.)	<b><math>\leq 100</math></b> (pcs.)	<b><math>\leq 150</math></b> (pcs.)	<b>&gt; 150</b> (pcs.)	<b>N</b>
<b>Clinical</b> human blood	<b>25</b>	<b>39</b>	<b>47</b> (52%)	<b>31</b> (34%)	<b>6</b> (7%)	<b>4</b> (4%)	<b>3</b> (3%)	<b>91</b>
<b>Artificial</b> Sheep blood	<b>56</b>	<b>26</b>	<b>2</b> (3%)	<b>29</b> (41%)	<b>35</b> (50%)	<b>4</b> (6%)	<b>0</b>	<b>70</b>

# Overall Cleaning Result of Clinical & Artificial Test Pieces



# Conclusion

- The quantity of residual protein was determined unexpectedly from artificial test pieces contaminated by sheep blood.
  - Further experiments will be followed to get a concrete conclusion, which include determination of the initial protein load on test pieces and modification of the loading position in WD.
- The quantity of residual protein of clinical test pieces was distributed from  $<20\mu\text{g}$  to  $174\mu\text{g}$ . 86% of clinical test pieces were gathered  $\leq 50\mu\text{g}$ .
  - The acceptance criteria of residual protein on a reprocessed instrument will be discussed and established according to the result.

Thank you very much!

