Design Verification

HumaTex RF Latex Agglutination Slide Test

Contents

1 Function

The HumaTex RF latex agglutination slide test has been designed as a qualitative and semi-quantitative test for the rapid determination of rheumatoid factors (RF) in native undiluted serum samples. The test is based on latex agglutination technique, featuring polystyrene latex which has been coated with human immunoglobulin G (IgG). If RF positive sera are reacted with the latex suspension, visible agglutinates will form within 2 minutes. Positive and negative controls are incorporated in the kit to perform function checks and to compare the results obtained with unknown samples. For semi-quantitative determinations the serum samples are diluted stepwise with glycine saline buffer. The last dilution step which give a positive result (agglutination) is used to calculate the RF concentration in the sample.

2 Reproducibility

The within-run reproducibility of the HumaTex RF (lot H2138, exp date 2004-11) latex agglutination slide test was calculated from 10-fold determinations of positive and negative samples. The day-to-day reproducibility was calculated from the results obtained on 4 different dates HumaTex RF (lot H2149, exp date 2005-09). Positive and negative control sera were employed as sample materials. HumaTex RF showed in all cases a very good reproducibility.

3 Sensitivity and Dynamic Range

Description of control materials

In-house standard are employed which have been calibrated against a standard material (Behringwerke, #OSHD 04/05). Standard dilutions are prepared by diluting the in-house standard with glycine buffered saline.
The following standard dilutions are prepared:

No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
RF, IU/ml	0	5	6	8	12	16	20

The kit controls positive and negative are employed for function test. The positive control is typically adjusted to a RF concentration of 40-50 IU/ml.

Preparation of Serum Control Panel

A panel of each 10 sera, RF positive and negative, has been established and confirmed with an independent test method. Each individual panel material has been aliquoted and is kept deep-frozen. Before use, the appropriate number of panel sera are removed from the freezer and brought to room temperature. Once thawed, panel sera must not be frozen again but have to be discarded after use.

Sensitivity Test Results

All concentrations are tested in double. The difference in agglutination degree must not exceed one titer step.

No.	**RF, IU/ml**	**Agglutination degree at 2 min.**
1	0	-
2	5	-
3	6	-
4	8	-
5	12	1+
6	16	2+
7	20	3+

Intensity grades: 4+ (very strong agglutination); 3+ (strong agglutination); 2+ (medium agglutination); 1+ (visible); ± (borderline); - (no agglutination)

Function Test with Kit Controls

The kit controls are employed for a function test of the latex reagent.

Controls	Agglutination degree at 2 minutes
Positive control	4+
1/2 diluted positive control	2+
1/4 diluted positive control	±
1/8 diluted positive control	-
Negative control	-

Specificity Check with In-house Panel Sera

Predetermined in-house panel sera have been employed for a specificity check.

No.	Positive panel	Negative panel
1	4+	-
2	4+	-
3	4+	-
4	2+	-
5	2+	-
6	2+	-
7	3+	-
8	4+	-
9	3+	-
10	3+	-

4 Interferences

Description of materials

Interferences have been tested for bilirubin, lipid, hemoglobin and ascorbic acid prepared in a human serum pool tested negative for CRP and RF. These serum preparations were spiked with RF resulting in a borderline agglutination (+/-).

Results

Substance	Serum pool w/o RF	Serum pool with RF
Serum pool (control)	neg	+/- to 1+
Bilirubin (40mg/dl)	neg	+/-
Lipids (1000mg/dl)	neg	+/-
Hemoglobin (500mg/dl)	neg	+/-
Ascorbic acid (500mg/dl)	neg	+/-

None of the above substances showed any influence on the expected results.

5 Specificity and Sensitivity

Description of method and specimen

The specificity and sensitivity of the HumaTex RF test has been evaluated by method comparison against the latex slide test Behring Rapitex. 61 native sera, 27 sera of healthy volunteers and 34 sera of rheumatoid patients, have been employed in this study.

Results

		Behring Rapitex RF Latex Test	
		Positive	Negative
HumaTex RF	Positive	33	2
	Negative	1	25
	Total	34	27

Of the 34 positive sera 33 were correctly found positive with the HumaTex RF test. The one sample which came up negative with the HumaTex RF test showed a concentration of 11 IU/ml in the quantitative test and was therefore near the detection limit of the test. 25 of 27 negative samples were correctly determined as negative with the HumaTex RF test. 2 sera produced borderline agglutination.

From the above a diagnostic specificity for the HumaTex RF test resulted to 92%.
The diagnostic sensitivity could be calculated to 97%.

6 Stability

Real-time Stability

The stability of the HumaTex RF latex agglutination slide test has been demonstrated on real-time stability studies and is checked by temperature stress studies on each produced lot.
A function tests on the finalised kit has been performed using standard materials and kit controls. Results of the function tests are summarised below.

Latex rgt. lot.: 046 Manuf. date: JUL. 94; Expiry date: 01-MAY 96

RF, IU/ml	20	16	12	8	6	0	neg. control	pos. control
Result fresh	4+	4+	3+	1+	-	-	-	4+
Result on 01/1996	4+	4+	3+	1+	-	-	-	4+
Result on 07/1996	4+	4+	3+	1+	-	-	-	4+

Latex rgt. lot.: 047 Manuf. date: AUG. 94; Expiry date: 01-JUN.96

RF, IU/ml	20	16	12	8	6	0	neg. control	pos. control
Result fresh	4+	3+	3+	1+	-	-	-	4+
Result on 01/1996	4+	3+	3+	1+	-	-	-	4+
Result on 07/1996	4+	3+	2+	1+	-	-	-	4+

Latex rgt. lot.: 048 Manuf. date: SEP. 94; Expiry date: 01-JUL. 96

RF, IU/ml	20	16	12	8	6	0	neg. control	pos. control
Result fresh	4+	4+	3+	1+	-	-	-	4+
Result on 01/1996	4+	4+	3+	1+	-	-	-	4+
Result on 07/1996	4+	4+	2+	1+	-	-	-	4+

Results

Three individual production batches of HumaTex RF have been checked after 23, 22 and 21 months real-time storage (storage conditions: 2...8°C). The above results clearly demonstrate the stability of HumaTex RF and support the stability claim of 18 months.