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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

RUBBER

UPOV Code: HEVEA

Hevea Aubl.

[*]

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by experts from Brazil

to be considered by the Enlarged Editorial Committee at its meeting to be held in Geneva, Switzerland, on January 8, 2009

Alternative Names:[*]

Botanical name	*English*	*French*	*German*	*Spanish*
Hevea Aubl.	Rubber	Hevea		Ule

The purpose of these guidelines ("Test Guidelines") is to elaborate the principles contained in the General Introduction (document TG/1/3), and its associated TGP documents, into detailed practical guidance for the harmonized examination of distinctness, uniformity and stability (DUS) and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

ASSOCIATED DOCUMENTS

These Test Guidelines should be read in conjunction with the General Introduction and its associated TGP documents.

[*] These names were correct at the time of the introduction of these Test Guidelines but may be revised or updated. [Readers are advised to consult the UPOV Code, which can be found on the UPOV Website (www.upov.int), for the latest information.]

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Hevea* Aubl.

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of a brown dormant bud grafted on a rootstock to be specified by the authority.

2.3 The minimum quantity of plant material, to be supplied by the applicant, should be:

10 plants.

2.4 The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

2.5 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

3. Method of Examination

3.1 Number of Growing Cycles

3.1.1 The minumum duration of tests should normally be a single growing cycle.

3.1.2 The growing cycle is considered to be the period ranging from the beginning of active vegetative growth, continuing through active vegetative growth and concluding with seed maturity. The growing cycle will be at least 5 years.

3.2 Testing Place

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 “Examining Distinctness”.

3.3 Conditions for Conducting the Examination

3.3.1 The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

3.3.2 The optimum stage of development for the assessment of each characteristic is indicated by a letter in the second column of the Table of Characteristics. The stages of development denoted by each letter are described in Chapter 8.1.

3.3.3 The recommended method of observing the characteristic is indicated by the following key in the second column of the Table of Characteristics:

MS: measurement of a number of individual plants or parts of plants
VG: visual assessment by a single observation of a group of plants or parts of plants
VS: visual assessment by observation of individual plants or parts of plants

3.4 Test Design

3.4.1. Each test should be designed to result in a total of at least 7 spaced plants.

3.4.2. The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

3.5 Number of Plants / Parts of Plants to be Examined

Unless otherwise indicated, all observations should be made on 5 plants or parts taken from each of 5 plants. In the case of parts of plants, the number to be taken from each of the plants should be 3.

3.6 Additional Tests

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 Distinctness

4.1.1 General Recommendations

It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

4.1.2 Consistent Differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear Differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative

manner. Therefore, it is important that users of these Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.2 Uniformity

4.2.1. It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:

4.2.2. For the assessment of uniformity of vegetatively propagated varieties, a population standard of 95% and an acceptance probability of at least 1% should be applied. In the case of a sample size of 7 plants, 1 off-type is allowed.

4.3 Stability

4.3.1 In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 Where appropriate, or in cases of doubt, stability may be tested, either by growing a further generation, or by testing a new plant stock to ensure that it exhibits the same characteristics as those shown by the previous material supplied.

5. Grouping of Varieties and Organization of the Growing Trial

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics:

(a) Trunk: axis (characteristic 15)
(b) Trunk: diameter (characteristic 16)
(c) Tree: beginning of wintering (characteristic 23)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction.

6. Introduction to the Table of Characteristics

6.1 Categories of Characteristics

6.1.1 Standard Test Guidelines Characteristics

Standard Test Guidelines characteristics are those which are approved by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.

6.1.2 Asterisked Characteristics

Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.

6.2 States of Expression and Corresponding Notes

States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.

6.3 Types of Expression

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the General Introduction.

6.4 Example Varieties

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

6.5 Legend

(*) Asterisked characteristic – see Chapter 6.1.2

QL: Qualitative characteristic – see Chapter 6.3
QN: Quantitative characteristic – see Chapter 6.3
PQ: Pseudo-qualitative characteristic – see Chapter 6.3

MS, VG, VS: See Chapter 3.3.3.

(a)-(c) See Explanations on the Table of Characteristics in Chapter 8.1
(+) See Explanations on the Table of Characteristics in Chapter 8.2

7. Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1. **(*)** **(+)**	**VG**	**Leaf cluster: shape of top**	**Touffe de feuilles : forme du sommet**	**Blattbüschel: Form der Spitze**	**Racimo de hojas: forma de la parte superior**		
PQ	**(a)**	acute	aigu	spitz	aguda	RRIC 102, RRIM 600, PB 235	1
		obtuse	obtus	stumpf	obtusa	IAN 717, TP 749	2
		round	rond	rund	redondeada	RRIC 100	3
		flattened	étalé	abgeflacht	achatada	GT1	4
2. **(*)**	**VG**	**Leaf: central leaflet shape compared to laterals**	**Feuille : forme de la foliole médiane par rapport aux folioles latérales**	**Blatt: Form des mittleren Fiederblatts im Vergleich zu den Seitenfiedern**	**Hoja: forma del foliolo central comparado con los laterales**		
QN		same or slightly different	identique ou légèrement différente	gleich oder etwas verschieden	la misma o ligeramente diferente	GT1	1
		moderately different	peu différente	mäßig verschieden	moderadamente diferente	PB 260	2
		very different	très différente	sehr verschieden	muy diferente	F 4512, FDR 5953	3
3. **(*)**	**VG**	**Leaf: intensity of green color of upper side**	**Feuille : intensité de la couleur verte de la face supérieure**	**Blatt: Intensität der Grünfärbung der Oberseite**	**Hoja: intensidad del color verde del haz**		
QN		light	claire	hell	claro	BPM 1, PB 235, RRIM 600	
		medium	moyenne	mittel	medio	BPM 24	5
		dark	foncée	dunkel	oscuro	GT1	7
4. **(*)**	**VG**	**Leaf: glossiness of upper side**	**Feuille : brillance de la face supérieure**	**Blatt: Glanz der Oberseite**	**Hoja: brillo del haz**		
QN	**(a)**	absent or weak	absente ou faible	fehlend oder gering	ausente o débil	BPM 24	1
		medium	moyenne	mittel	medio	GT1, RRIM 600	2
		strong	forte	stark	fuerte	PA 31	3

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
5. **(*)**	**VG**	**Leaf: surface of upper side**	**Feuille : surface de la face supérieure**	**Blatt: Oberfläche der Oberseite**	**Hoja: superficie del haz**		
QN		smooth or slightly rough	lisse ou légèrement rugueuse	glatt oder leicht rauh	lisa o ligeramente rugosa	PB 235, PB 260	1
		moderately rough	peu rugueuse	mäßig rauh	moderadamente rugosa	GT1, RRIM 600	2
		very rough	très rugueuse	sehr rauh	muy rugosa	RRIC 101	3
6.		**Leaf: pubescence on veins on lower side**	**Feuille : pubescence sur les nervures de la face inférieure**	**Blatt: Behaarung an den Adern an der Unterseite**	**Hoja: pubescencia en los nervios del envés**		
QL	**(a)**	absent	absente	fehlend	ausente	PB 235, RRIM 600	1
		present	présente	vorhanden	presente	F 4542, RRIC 101	9
7. **(+)**	**VG**	**Leaflet blade: attitude in relation to petiole**	**Limbe de la foliole : port par rapport au pétiole**	**Fiederblattspreite: Haltung im Verhältnis zum Blattstiel**	**Limbo del foliolo: actitud en relación con el pecíolo**		
QN	**(a)**	semi-erect	demi-dressé	halbaufrecht	semierecto	FDR 5788	1
	(b)	horizontal	horizontal	waagerecht	horizontal	RRIC 100	2
		semi-drooping	demi-retombant	überhängend	semicolgante	IRCA 41, PA31	3
8.	**VG**	**Leaflet blade: length**	**Limbe de la foliole : longueur**	**Fiederblattspreite: Länge**	**Limbo del foliolo: longitud**		
QN	**(a)**	short	court	kurz	corto	FDR 4151	3
	(b)	medium	moyen	mittel	medio	GT1, PB 217, PB 235, RRIM 600	5
		long	long	lang	largo	RRIC 100	7
9. **(*)** **(+)**	**VG**	**Leaflet blade: position of broadest part**	**Limbe de la foliole : position de la partie la plus large**	**Fiederblattspreite: Position des breitesten Teils**	**Limbo del foliolo: posición de la parte más ancha**		
QN	**(a)**	towards base	vers la base	zur Basis hin	hacia la base		1
	(b)	at middle	au milieu	in der Mitte	en el medio	PB 217, RRIM 703	2
		towards apex	vers le sommet	zur Spitze hin	hacia el ápice	RRIM 600	3

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
10. **(*)** **(+)**	**VG**	**Leaflet blade: axis in longitudinal section**	**Limbe de la foliole : axe en section longitudinale**	**Fiederblattspreite: Achse im Längsschnitt**	**Limbo del foliolo: eje en la sección longitudinal**		
PQ	**(a)**	straight	droit	gerade	recto	BPM1	1
	(b)	convex	convexe	konvex	convexo	GT1	2
		sigmoid	sigmoïde	S-förmig	sigmoideo	PB 260	3
11. **(*)** **(+)**	**VG**	**Leaflet blade: undulation of margin**	**Limbe de la foliole : ondulation du bord**	**Fiederblattspreite: Randwellung**	**Limbo del foliolo: ondulación del borde**		
QN	**(a)**	absent or weak	absente ou faible	fehlend oder gering	ausente o débil	BPM 24, PB 235, RRIM 600	1
	(b)	medium	moyenne	mittel	media	GT1, PB 260, RRIC 100	2
		strong	forte	stark	fuerte	RRII5, RRII118, RRIM701	3
12. **(+)**	**VG**	**Leaflet blade: shape of base**	**Limbe de la foliole : forme de la base**	**Fiederblattspreite: Form der Basis**	**Limbo del foliolo: forma de la base**		
PQ	**(a)**	attenuate	effilée	verjüngt	afilada		1
	(b)	cuneate	cunéiforme	keilförmig	cuneiforme		2
		obtuse	obtuse	stumpf	obtusa		3
13. **(+)**	**VG**	**Leaflet blade: shape of apex excluding tip**	**Limbe de la foliole : forme du sommet (pointe exclue)**	**Fiederblattspreite: Form der Spitze ohne aufgesetzte Spitze**	**Limbo del foliolo: forma del ápice, excluida la punta**		
PQ	**(a)**	acute	aiguë	spitz	agudo	FDR 5332, F 4512, PB 235, RRII105	1
	(b)	obtuse	obtuse	stumpf	obtuso	FDR 5203, PB 260, RRIM 600	2
		rounded	arrondie	abgerundet	redondeado	FDR 5731	3

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
14. **(*)** **(+)**	**VG**	**Petiole: attitude**	**Pétiole : port**	**Blattstiel: Haltung**	**Pecíolo: porte**		
QN		semi-erect	demi-dressé	halbaufrecht	semierecto	CDC 25, GT1, RRIC 100, RRIM 600, RRIM 703	
		horizontal	horizontal	waagerecht	horizontal	CDC 343, PB 235, PB 260	
		semi-drooping	demi-retombant	überhängend	semicolgante	MDX 571	
15. **(*)** **(+)**		**Trunk: axis**	**Tronc : axe**	**Stamm: Achse**	**Tronco: eje**		
QN		straight or slightly curved	droit ou légèrement courbé	gerade oder leicht gebogen	recto o ligeramente curvado	CDC 312, FDR 5788, GT1, RRIM 600	
		moderately curved	peu courbé	mäßig gebogen	moderadamente curvado	RRII5	
		strongly curved	très courbé	stark gebogen	muy curvado	TP 875	
16. **(*)** **(+)**		**Trunk: diameter**	**Tronc : diamètre**	**Stamm: Durchmesser**	**Tronco: diámetro**		
QN	**(c)**	small	petit	klein	pequeño	PR 107	3
		medium	moyen	mittel	medio	GT1, RRIM 600	5
		large	grand	groß	grande	CDC 312, PB 235, PB 260	7
17. **(+)**	**VG**	**Trunk: main color of bark**	**Tronc : couleur principale de l'écorce**	**Stamm: Hauptfarbe der Rinde**	**Tronco: color principal de la corteza**		
PQ	**(c)**	reddish brown	brun rougeâtre	rötlichbraun	marrón rojizo	PB 314	1
		brown	brun	braun	marrón	PB 217, PB 312, RRIM 600	2
		grey	gris	grau	gris	PB 235	3

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
18.	**VG**	**Trunk: texture of bark**	**Tronc : texture de l'écorce**	**Stamm: Textur der Rinde**	**Tronco: textura de la corteza**		
QN	**(c)**	smooth or slightly rough	lisse ou légèrement rugueuse	glatt oder leicht rauh	lisa o ligeramente rugosa	FDR 5788, PB 235	1
		moderately rough	peu rugueuse	mäßig rauh	moderadamente rugosa	GT1	2
		very rough	très rugueuse	sehr rauh	muy rugosa	CDC 308	3
19.	**VG**	**Tree: shape**	**Arbre : forme**	**Baum: Form**	**Árbol: forma**		
(+)							
PQ	**(c)**	triangular	triangulaire	dreieckig	triangular	PB 217, PB 235, PB 260	1
		ovate	ovale	eiförmig	oval		2
		circular	circulaire	rund	circular	PB 314	3
		oblate	aplatie	breitrund	achatada	RRIM 600	4
20. **(*)**	**VG**	**Tree: density of foliage**	**Arbre : densité du feuillage**	**Baum: Dichte des Laubes**	**Árbol: densidad del follaje**		
QN	**(c)**	sparse	faible	locker	ralo	FDR 5788, PR 261	3
		medium	moyenne	mittel	medio	PB 260	5
		dense	forte	dicht	denso	PA 31, PB 217, PB 314	7
21. **(*)** **(+)**	**VG**	**Coagulum: color of surface**	**Coagulum : couleur de la surface**	**Coagulum: Farbe der Oberfläche**	**Coágulo: color de la superficie**		
PQ	**(c)**	white	blanc	weiß	blanco	GT1, PB 217, RRIM 600	1
		light yellow	jaune clair	hellgelb	amarillo claro	PB260	2
		medium yellow	jaune moyen	mittelgelb	amarillo medio		3
		dark grey	gris foncé	dunkelgrau	gris oscuro	IAN 3156, RRII 203	4
22. **(*)** **(+)**	**VG**	**Tree: wintering**	**Arbre : hivernage**	**Baum: Winterruhe**	**Árbol: defoliación**		
QN		absent	absent	fehlend	ausente	PA 31	1
		partial	partiel	partiell	parcial	F 4512, GT1	2
		full	total	vollständig	total	PB 260, RRIM 600	3

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
23. (*)	**VG**	**Tree: beginning of wintering**	**Arbre : début d'hivernage**	**Baum: Beginn der Winterruhe**	**Árbol comienzo de la defoliación**		
QN	**(c)**	early	précoce	früh	temprana	BPM 1, PB 260	3
		medium	moyen	mittel	media	PB 235	5
		late	tardif	spät	tardía	GT1, RRIM 600	7
24. (*) (+)	**MG**	**Seed: length**	**Graine : longueur**	**Samen: Länge**	**Semilla: longitud**		
QN		short	courte	kurz	corta	GT1	
		medium	moyenne	mittel	media	RRIM 600	
		long	longue	lang	larga	CDC 312, RRIC 100	
25. (*) (+)		**Seed: width**	**Graine : largeur**	**Samen: Breite**	**Semilla: anchura**		
QN	**(c)**	narrow	étroite	schmal	estrecha	GT1	1
		medium	moyenne	mittel	media	RRIM 600	2
		broad	large	breit	ancha	RRIC 100	3
26. (*) (+)	**MG**	**Seed: thickness**	**Graine : épaisseur**	**Samen: Dicke**	**Semilla: grosor**		
QN	**(c)**	thin	mince	dünn	delgada	PB 260, RRIM 600	1
		medium	moyenne	mittel	media	IRCA 317, PB 235, PB 280	2
		thick	épaisse	dick	gruesa	CDC 312, RRIC 100	3
27. (*) (+)	**VG**	**Seed: shape in dorsal view**	**Graine : forme en vue dorsale**	**Samen: Form in Rückenansicht**	**Semilla: forma en vista dorsal**		
PQ	**(c)**	elliptic	elliptique	elliptisch	elíptica	FDR 233, PB 235	1
		circular	circulaire	rund	circular	IRCA 339, RRIM 600	2
		oblong	oblongue	länglich	oblonga	FDR 18, RRII 105	3
		obovate	obovale	verkehrt eiförmig	oboval	IRCA 621, RRIM 623	4

8. Explanations on the Table of Characteristics

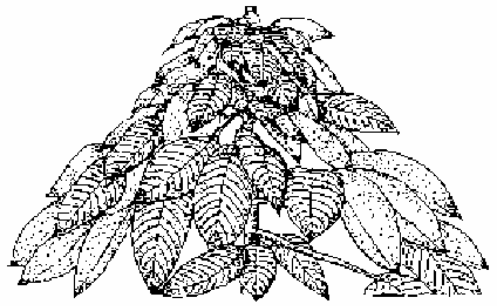
8.1 Explanations covering several characteristics

Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

(a) observations should be made on young plants, approximately 18 months old (last flush of mature leaves)
(b) observation should be made on the central leaflet
(c) observations should be made on mature trees with a fully developed trunk, approximately 5 years old

8.2 Explanations for individual characteristics

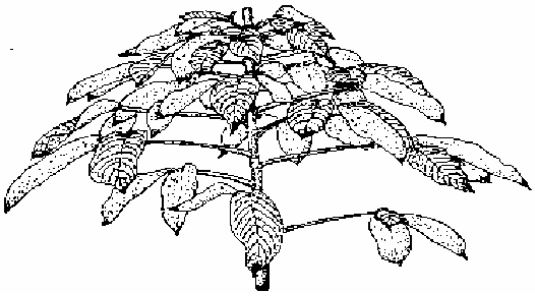
Ad. 1: Leaf cluster: shape of top



1
acute

2
obtuse

3
round



4
flattened

Ad. 7: Leaflet blade: attitude in relation to petiole

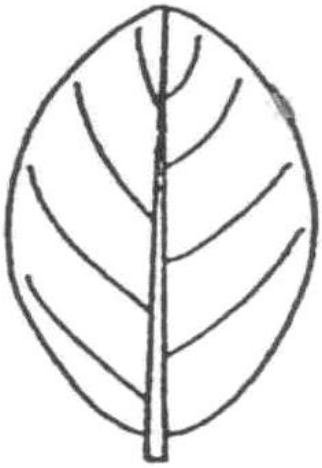
1
semi-erect

2
horizontal

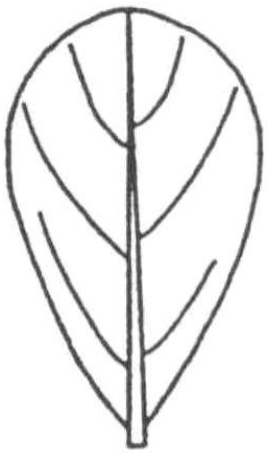
3
semi-drooping

Ad. 9: Leaflet blade: position of broadest part

1
towards base

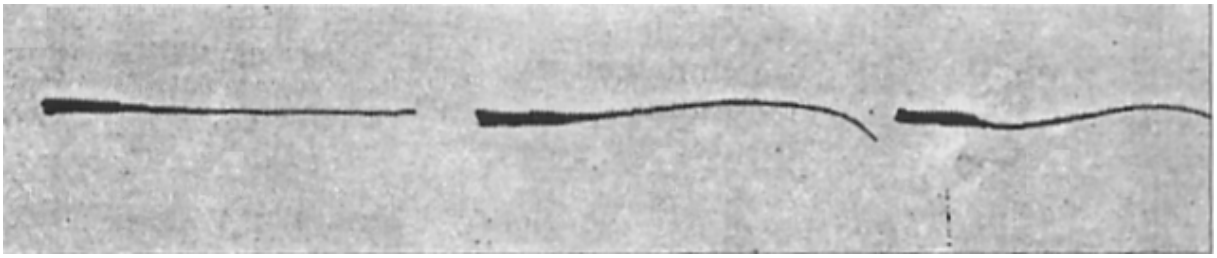


2
at middle



3
towards apex

Ad. 10: Leaflet blade: axis in longitudinal section

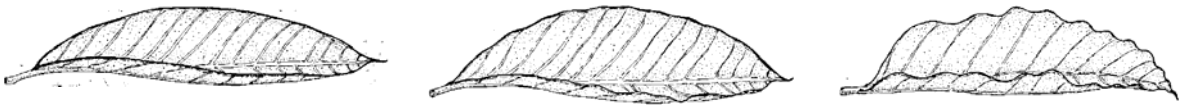


1
straight

2
convex

3
sigmoid

Ad. 11: Leaflet blade: undulation of margin

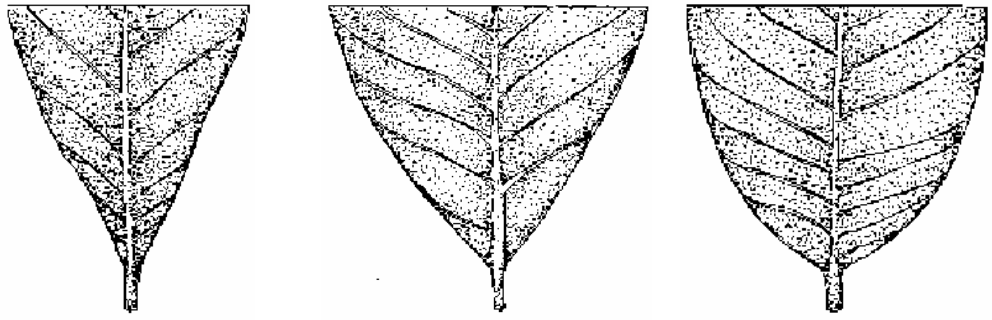


1
absent or weak

2
medium

3
strong

Ad. 12: Leaflet blade: shape of base

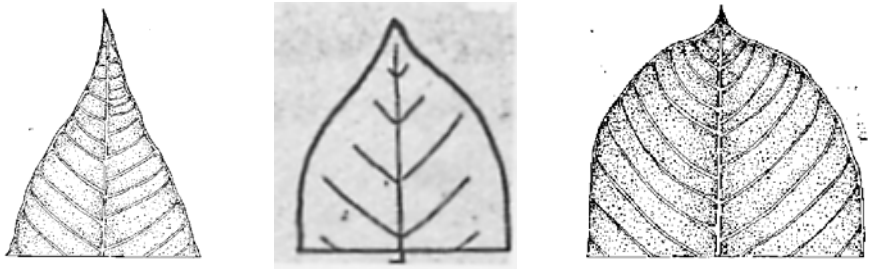


1
attenuate

2
cuneate

3
obtuse

Ad. 13: Leaflet blade: shape of apex excluding tip

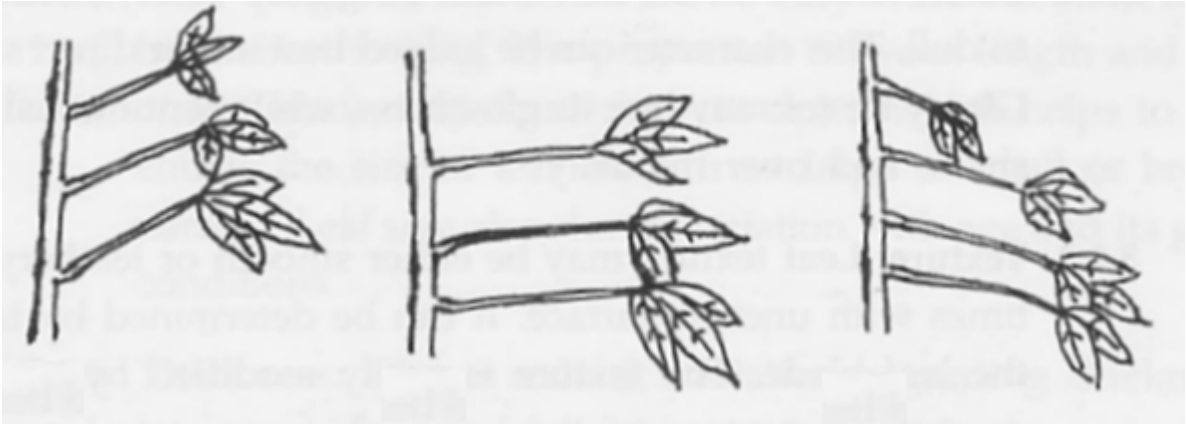


1
acute

2
obtuse

3
rounded

Ad. 14: Petiole: attitude

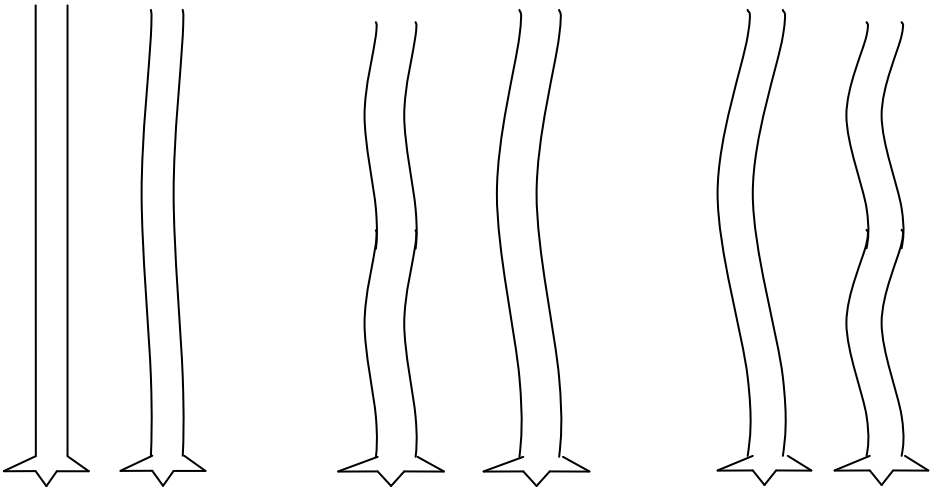


1
semi-erect

2
horizontal

3
semi-drooping

Ad. 15: Trunk: axis



1	2	3
straight or slightly curved	moderately curved	strongly curved

Ad. 16: Trunk: diameter

The diameter of the trunk should be observed at 1 meter above the ground.

Ad. 17: Trunk: main color of bark

The main color is the color with the largest surface area.

Ad. 19: Tree: shape

1
triangular

2
ovate

3
circular

4
oblate

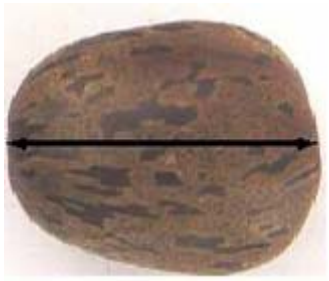
Ad. 21: Coagulum: color of surface

Observation of color is made on 5 ml of latex collected in a plastic cup from a tree tapped in half-spirale from at least one month. The coagulum color is evaluated on the surface exposed to the air, 48 hours after tapping.

Ad. 22: Tree: wintering

Absent: no leaves fall
Partial: some leaves fall
Full: all leaves fall from the tree

Ad. 24: Seed: length



Ad. 25: Seed: width

Ad. 26: Seed: thickness

Ad. 27: Seed: shape in dorsal view

1
elliptic

2
circular

3
oblong

4
obovate

9. Literature

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10. Technical Questionnaire

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

	Application date: (not to be filled in by the applicant)

TECHNICAL QUESTIONNAIRE
to be completed in connection with an application for plant breeders' rights

1. Subject of the Technical Questionnaire

1.1 Genus	
1.1.1 Botanical name	*Hevea* Aubl.
1.1.2 Common name	Rubber
1.2 Species (please complete)	

2. Applicant

Name	
Address	
Telephone No.	
Fax No.	
E-mail address	
Breeder (if different from applicant)	

3. Proposed denomination and breeder's reference

Proposed denomination (if available)	
Breeder's reference	

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]
(please state parent varieties)
(b) partially known cross [ ]
(please state known parent variety(ies))
(c) unknown cross [ ]

4.1.2 Mutation [ ]
(please state parent variety)

4.1.3 Discovery and development [ ]
(please state where and when discovered
and how developed)

4.1.4 Other [ ]
(please provide details)

4.2 Method of propagating the variety

4.2.1 Vegetative propagation

(a) bud grafting [ ]

(b) cuttings [ ]

(c) *in vitro* propagation

(d) other (state method) [ ]

4.2.2 Other [ ]
(please provide details)

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).

	Characteristics	Example Varieties	Note
5.1 (15)	**Trunk: axis**		
	straight or slightly curved	CDC 312, FDR 5788, GT1, RRIM 600	1[ ]
	moderately curved	RRII5	2[ ]
	strongly curved	TP 875	3[ ]
5.2 (16)	**Trunk: diameter**		
	small	PR 107	3[ ]
	medium	GT1, RRIM 600	5[ ]
	large	CDC 312, PB 235, PB 260	7[ ]
5.3 (23)	**Tree: beginning of wintering**		
	early	BPM 1, PB 260	3[ ]
	medium	PB 235	5[ ]
	late	GT1, RRIM 600	7[ ]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

6. Similar varieties and differences from these varieties

Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the **similar** variety(ies)	Describe the expression of the characteristic(s) for **your** candidate variety
Example	*Trunk: texture of bark*	*smooth*	*moderately rough*

Comments:

[#]7. Additional information which may help in the examination of the variety

7.1 In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?

Yes [ ] No [ ]

(If yes, please provide details)

7.2 Are there any special conditions for growing the variety or conducting the examination?

Yes [ ] No [ ]

(If yes, please provide details)

7.3 Other information

[#] Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

8. Authorization for release

(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes [ ] No [ ]

(b) Has such authorization been obtained?

Yes [ ] No [ ]

If the answer to (b) is yes, please attach a copy of the authorization.

9. Information on plant material to be examined or submitted for examination.

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

(a) Microorganisms (e.g. virus, bacteria, phytoplasma) Yes [ ] No [ ]

(b) Chemical treatment (e.g. growth retardant, pesticide) Yes [ ] No [ ]

(c) Tissue culture Yes [ ] No [ ]

(d) Other factors Yes [ ] No [ ]

Please provide details for where you have indicated “yes”.

……

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant’s name

Signature Date

[Annex follows]

ANNEX

Additional Useful Explanations

TABLE OF CONTENTS PAGE

Part I

Introduction

The following Annex contains a list of characteristics derived by using electrophoresis and a description of the method to be used. UPOV decided to place these characteristics in a Annex to establish the Test Guidelines, thereby creating a special category of characteristic, because the majority of the UPOV member States is of the view that it is not possible to establish distinctness solely on the basis of a difference found in a characteristic derived by using electrophoresis. Such characteristics should therefore be used as a complement to other differences in morphological or physiological characteristics. UPOV reconfirms that these characteristics are considered useful but that they might not be sufficient on their own to establish distinctness. They should not be used as a routine characteristic but at the request or with the agreement of the applicant of the candidate variety.

Procedure described hereafter can be particularly carried out according to the specifications made by CIRAD for *Hevea* genus.(Leconte et al., 1994, *Electrophoresis application to Hevea clone identification*, Plantations, Recherche, Développement, 2:28-36 – Leconte 1997, *Starch gel electrophoresis for rubber clone identification. Practical handbook*). At its maximum, the analyses of the 13 enzymatic systems can be carried out simultaneously in the same day by using 2 starch gels respectively with pH6.6 and pH8. Analysis can also be focussed only on some of the 13 available enzymatic systems depending on the varieties being analysed.

For each enzymatic system, there are a number of different possible alleles at each locus and the analysis of each isoform is based on the recognition of the alleles from these proteins, which appear on gels as a series of well-defined bands or patterns of bands. The alleles are described by band numbers according to the definition given to them by Cirad

Part II

Characteristics Derived by Using Electrophoresis

	Characteristics Caractères	English	Français	Example Varieties Exemples	Note
28.	Malate dehydrogenase composition	*band 1*	*bande 1*		1
		band 1 + 2	*bande 1 + 2*	CD 1078	2
		band 1 + 3	*bande 1 + 3*	IAN 3087	3
(+)	Composition de la malate déshydrogenase	*band 1 + 4*	*bande 1 + 4*	F 4542	4
		band 2	**bande 2**	GT1, PB 235	5
		band 2 + 3	**bande 2 + 3**	BPM 24, PB 260	6
		band 2 + 4	bande 2 + 4	FX 3899	7
		band 3	bande 3	PB 217, IRCA 111	8
		band 3 + 4	*bande 3 + 4*	SCH P 52	9
		band 4	*bande 4*	F 4506	10
29.	Phospho glucose isomerase composition	**band 1**	**bande 1**	PB 260, PB 217, PB 260	1
		band 1 + 2	**bande 1 + 2**	RRIM 519	2
		band 1 + 3	**bande 1 + 3**	GT1	3
(+)		band 1 + 4	bande 1 + 4	AC 58	4
	Composition de la phospho glucose isomérase	band 1 + 5	bande 1 + 5	IRCA 130	5
		band 2	bande 2	RO 51	6
		band 2 + 3	**bande 2 + 3**	PB 235	7
		band 2 + 4	*bande 2 + 4*	BPM 22	8
		band 2 + 5	*bande 2 + 5*	GU 174	9
		band 3	**bande 3**	PB 314	10
		band 3 + 4	bande 3 + 4	AC 53	11
		band 3 + 5	*bande 3 + 5*	RRIM 527	12
		band 4	*bande 4*	F 4542	13
30.	Alanine amino peptidase composition	*band 1 + 2*	*bande 1 + 2*	RO 53	1
		band 2	**bande 2**	GT1, PB 217, PB 260, PB 235	2
		band 2 + 3	*bande 2 + 3*	RO 58	3
(+)	Composition de l'alanine amino peptidase	band 2 + 4	bande 2 + 4	RRIC 130	4
		band 3	*bande 3*	RO 60	5
		band 3 + 4	*bande 3 + 4*	RO 61	6
		band 4	*bande 4*	F 4506	7
31.	Leucine amino peptidase composition	band 1 + 2	bande 1 + 2	FX 3899	1
		band 1 + 6	*bande 1 + 6*	RO 51	2
		band 2	**bande 2**	BPM 24, GT1,PB 217,PB 235	3
(+)	Composition de la leucine amino peptidase	*band 2 + 3*	*bande 2 + 3*	PB 5/63	4
		band 2 + 4	**bande 2 + 4**	PB 260	5
		band 2 + 5	bande 2 + 5	RRIC 100	6
		band 2 + 6	bande 2 + 6	RRIC 101	7
		band 2 + 7	*bande 2 + 7*	WAR 4	8
		band 4	bande 4	PB 312	9
		band 4 + 6	bande 4 + 6	RRIC 132	10
		band 4 + 7	*bande 4 + 7*	PA 31	11
		band 5	*bande 5*		12
		band 6	bande 6	MDF 180	13
		band 6 + 7	*bande 6 + 7*	MDF 372	14
		band 6 + 8	*bande 6 + 8*	AC 60	15

	Characteristics Caractères	English	Français	Example Varieties Exemples	Note
32. (+)	Esterase composition Composition de l'estérase	**band 1**	**bande 1**	GT1	1
		band 1 + 2	*bande 1 + 2*	RO 58	2
		band 1 + 3	*bande 1 + 3*	AC 57	3
		band 1 + 4	bande 1 + 4	IRCA 621	4
		band 1 + 5	bande 1 + 5	FX 985	5
		band 1 + 6	**bande 1 + 6**	BPM 24, PB 217, PB 260	6
		band 1 + 7	*bande 1 + 7*	IAN 6590	7
		band 2 + 4	*bande 2 + 4*	RO 50	8
		band 3	*bande 3*	AC 53	9
		band 3 + 4	*bande 3 + 4*		10
		band 4	*bande 4*	F 4542	11
		band 4 + 5	*bande 4 + 5*	PA 31	12
		band 4 + 6	bande 4 + 6	FX 3899	13
		band 5	*bande 5*	F 4512	14
		band 5 + 6	bande 5 + 6	RRIC 121	15
		band 6	**bande 6**	PB 235	16
		band 6 + 7	*bande 6 + 7*	IAN 6587	17
33. (+)	Diaphorase composition Composition de la diaphorase	*band 1*	*bande 1*	F 4512	1
		band 1 + 2	*bande 1 + 2*	MDF 114	2
		band 1 + 3	*bande 1 + 3*	PFB5	3
		band 2	**bande 2**	IAN717	4
		band 2 + 3	bande 2 + 3	PilA 44	5
		band 2 + 6	*bande 2 + 6*	SCH C 133	6
		band 3	**bande 3**	GT 1, BPM 24, PB 217	7
		band 3 + 4	*bande 3 + 4*	AC80	8
		band 3 + 5	*bande 3 + 5*	MDF 362	9
		band 3 + 6	*bande 3 + 6*	SCH C 814	10
		band 4	*bande 4*	F 4542	11
		band 5	*bande 5*	IAN 6158	12
		band 5 + 6	*bande 5 + 6*	CNSAM 7706	13
34. (+)	Phosphatase acid composition Composition de la phosphatase acide	*band 0*	*bande 0*	P 122	1
		band 0 + 1	*bande 0 + 1*	PA 31	2
		band 1	**bande 1**	GT1,BPM24, PB 217, PB 235	3
		band 1 + 2	**bande 1 + 2**	RRIM 600	4
		band 2	bande 2	PB 235	5
35. (+)	Alcool dehydrogenase composition Composition de l'alcool déshydrogénase	band 2	bande 2	PB 310	1
		band 2 + 3	**bande 2 + 3**	RRIM 600, PB 235	2
		band 2 + 4	*bande 2 + 4*	RRIC 132	3
		band 3	**bande 3**	GT1, PB 217, PB 260	4
		band 3 + 4	bande 3 + 4	FX 3899	5
		band 4	*bande 4*	F 4542	6
36. (+)	Isocitrate dehydrogenase composition Composition de l'isocitrate déshydrogénase	*band 1 + 2*	*bande 1 + 2*	IAN717	1
		band 1 + 3	bande 1 + 3	FX 3899	2
		band 2	*bande 2*	RRIC 103	3
		band 2 + 3	bande 2 + 3	AC 53	4
		band 2 + 4	*bande 2 + 4*	CNSAM 7701	5
		band 3	**bande 3**	GT1	6
		band 3 + 4	**bande 3 + 4**	RRIM 600, PB 260, PB 235	7
		band 4	**bande 4**	PB 217	8
		band 4 + 5	*bande 4 + 5*	TU 45/525	9
37. (+)	Phosphogluconate dehydrogenase composition Composition de la phosphogluconate déshydrogénase	*band 1*	*bande 1*	RRIC 22	1
		band 1 + 2	**bande 1 + 2**	FX 3899	2
		band 1 + 3	**bande 1 + 3**	GT1, PR 107	3
		band 2	**bande 2**	AVROS 2037, PB 217	4
		band 2 + 3	**bande 2 + 3**	BPM 24, IAN 873	5
		band 3	**bande 3**	PFB 5	6

	Characteristics Caractères	English	Français	Example Varieties Exemples	Note
38. (+)	Phosphoglucomutase composition Composition de la phosphoglucomutase	*band 1 + 2*	*bande 1 + 2*	AC 80	1
		band 2	bande 2	AC 53	2
		band 2 + 3	bande 2 + 3	RO 60	3
		band 2 + 4	bande 2 + 4	IRCA 652	4
		band 3	bande 3	AC 62	5
		band 3 + 4	bande 3 + 4	IAN 710, RRIM 725	6
		band 3 + 5	*bande 3 + 5*	RRIM 729	7
		band 4	**bande 4**	RRIM600,GT1,PB260,PB217	8
		band 4 + 5	bande 4 + 5	RRIC 110	9
		band 4 + 6	*bande 4 + 6*	PUA 8	10
		band 5	*bande 5*	AVROS 152	11
39. (+)	Glutamate oxaloacetate transaminase composition Composition de la glutamate oxaloacétate transaminase	*band 0*	*bande 0*	SCH P 48	1
		band 1	**bande 1**	RRIM600,GT1,PB217,PB260	2
		band 1 + 2	**bande 1 + 2**	RRIC 110, PB 86, PB 235	3
		band 1 + 3	bande 1 + 3	RO 50	4
		band 2	bande 2	IRCA 707	5
40. (+)	Shikimate dehydrogenase composition Composition de la shikimate déshydrogénase	*band 1*	*bande 1*	AC 54	1
		band 1 + 3	bande 1 + 3	IRCA 37	2
		band 1 + 4	*bande 1 + 4*		3
		band 1 + 6	*bande 1 + 6*	GU 969	4
		band 2	*bande 2*	RO 55	5
		band 2 + 3	bande 2 + 3	PA 31, AVROS 152	6
		band 2 + 5	*bande 2 + 5*	CNSAM 7621	7
		band 3	**bande 3**	RRIM600,GT1,PB217,PB260	8
		band 3 + 4	bande 3 + 4	IRCA 621, RRIC 132	9
		band 3 + 5	bande 3 + 5	RO 46	10
		band 3 + 6	*bande 3 + 6*	FX 25	11
		band 4	*bande 4*	F 4512	12
		band 4 + 5	*bande 4 + 5*	F 4542	13
		band 5	*bande 5*	AC 68	14
		band 5 + 6	*bande 5 + 6*		15

Isoforms most abundant are in bold; Isoforms most rare are in italic.

Part III

Description of the Method to be Used

Starch gel electrophoresis for rubber clone identification

1. Apparatus and equipment

Any suitable horizontal electrophoresis system can be used. Specific gel moulds have been elaborated by Cirad for allowing the analysis of 13 enzymatic systems in one same process during a one-day time period. These moulds have been adapted in a bridge shape with holes so that both edges of the gel can be dipped in the buffer of electrode vessels for circulation of electric current through the gel.

2. Chemicals

All chemicals should be of 'Analytical Reagent' grade or better. The list of the products is given in one table at the end of this document.

3. Solutions

3.1 Extraction solutions

The extraction buffer has to be prepared the day of the analysis or the day before (conservation in the fridge)

Tris (TRIZMA BASE)	424 mg
Cystein	60 mg
Distilled water up to	40 ml

Adjust pH with HCl 1N solution (7.2<pH<7.5)
Complete to 50 ml.

Keep it under cool conditions.

3.2 Electrophoresis (running buffer)

Tris-Citrate pH 6.6:

Tris	18.2 g
Citric acid	10.5 g
Distilled water up to	1000 ml

Control pH (approximately 6.6)

Tris-Citrate pH 8:

Tris	18.6 g
Citric acid	8.4 g
Distilled water up to	1000 ml

Control pH (approximately 8)

3.3 Gel preparation solutions

Tris-histidin pH 6:
Histidin 5.25 g
Distilled water up to 400ml
Titrate to pH 6 with Tris 1M
Adjust level with distilled water up to 500 ml

Tris-histidin pH 8:
Histidine 5.10 g
Distilled water up to 400ml
Titrate to pH 8 with Tris 1M
Adjust level with distilled water up to 500 ml

3.4 Staining buffers

Tris HCl 0.5M pH 8.5
Tris 60.6 g
Distilled water up to 800 ml
Titrate to pH 8.5 with HCl 1N
Adjust level with distilled water up to 1000ml

Phosphate 0.1M pH 6.5
Na_2HPO_4, $2H_2O$ 3.8 g
KH_2PO_4 7.8 g
Distilled water up to 1000 ml

Acetate 0.5M pH 5
Sodium acetate 28.9 g
Acetic acid 8.5 ml
Distilled water up to 800 ml
Titrate to pH 5.4 with NaOH 10N
Adjust level with distilled water up to 1000ml

Tris maleate 0.1M pH 5.4
Tris 12.1 g
Maleic acid 11.6 g
Distilled water up to 800 ml
Titrate to pH 5.4 with NaOH 10N
Adjust level with distilled water up to 1000ml

3.5 Substrate solutions

Na malate 1M pH 7
DL malic acid 6.7 g
Distilled water 40 ml
Titrate to pH 7 with NaOH 10N
Adjust level with distilled water up to 50 ml

Leucine
Leucine β-Naphtyl amide HCl 250 mg

Dissolved in 100 ml distilled water (**Caution**: Leucine is very toxic, gloves are obligatory)

α-Naphtyl acetate / acetone

α-Naphtyl acetate	300 mg
Acétone	60 ml

3.6 Co-factor solutions

NAD+	10 mg / ml H_20
NADP+	10 mg / ml H_20

$MgCl_2$ 0.4M

$MgCl_2$, 6 H_20	8.1 g
Distilled water up to	100 ml

3.7 Enzyme solution

G6PDH Glucose 6 phosphate dehydrogenase: 1000 units / 250 µl ready to use

3.8 Staining solutions

PMS	Phenazine methosulfate	1 mg /ml
MTT	Dimethylthiazol tetrazolium	5 mg / ml
DCPIP	Dichlorophenol indophenol	1 mg /ml

4. Procedure

4.1 Protein extraction

Enzymes can be rapidly destroyed by moderately hot temperatures. Preservation must be applied by ensuring cold conditions, or by freeze-drying of the leaf samples soon after collection.

Three anthocyanic leaflets (brown-redish = B stage) are collected and immediately kept in fresh conditions in a cool box with ice. For best results, time between sampling and extraction should be shortened. At the laboratory, preparation of leaves and extraction may occur in cold conditions (air conditionned room and/or ice bed). Take out mortars and pestles kept cooled in a fridge since day before. In each mortar, add 20 mg PVPP (Polyvinyl Polypyrrolidone insoluble), 50 mg fresh leaflets (discard petiolus), 0.5-0.6 ml extraction buffer. Crush the whole with a pestle up to obtain an homogeneous mixture. On each mortar, place 1 slip blotting paper (one layer) and 2 pieces of Whatman paper n°3 (1.0 x 0.7 cm).

4.2 Preparation of the gels

The gels are prepared the day before. One single gel allows to visualise 6 or 7 enzymatic systems (alanine amino peptidase and leucine amino peptidase are jointly visualised). Prepare 2 gels: 1 gel at pH6 (Tris-histidin buffer, pH 6), and 1 gel at pH8 (Tris-histidin buffer, pH 8).

Obturate the holes of the two gel moulds with adhesive tape. Place each mould on a level table and adjust horizontality. In a 1-litre Büchner flask weigh 62.0 g of starch (12% gel). Add 50 ml gel buffer (pH6 or pH8), complete with 450 ml of distilled water. Add a 80-mm magnetic stirring rod. Obturate the flask with a mortar in the aim to avoid evaporation of water. Heat the suspension (300°C) under continuous stirring up to the boiling point (apparition of big bubbles). Cooking one gel needs around 15 to 20 min. The starch paste obtained is degassed in the Büchner flask for 2 – 3 minutes. This starch paste is then carefully poured into the first mould in avoiding the formation of air bubbles. Repeat these operations for the second gel. Allow starch polymerization to take place at room temperature (1h30) and then cover with a plastic film to avoid dessication. Let the plastic-covered gels at room temperature overnight, then put them at 4°C, 30 min before use.

Using a guide and a spatula (with thin edge), 26 slots are made in each gel, on a line located at a distance of 4 cm from one edge of the mould (26 different samples can be analysed for the 13 enzymatic systems over the two gels). This starting line of migration is marked with bromophenol blue solution. With thin tweezers, each Whatman piece of paper bearing the extract of one sample is inserted in its corresponding slot; for each sample, one Whatman piece of paper is inserted in the gel pH6, and a second one bearing the same extract is inserted in the gel pH8. After sample loading, gels are covered again with transparent plastic film, and adhesive tapes are removed from the moulds.

4.3 Electrophoresis

Migration is carried out under cool conditions (cool room of fridge). The two edges of each gel in its mould are placed in the two electrode vessels filled with appropriate buffer: Tris-Citrate pH 6.6 for gel pH6 and Tris-Citrate pH 8 for gel pH8. Migration is carried out at a constant amperage (50mA for one gel). Migration is over when bromophenol blue reaches the anode vessel, which takes 5 to 6 hours.

4.4 Slicing the gels

The cathodic and anode edges of the gel are removed first by cutting the cathode edge at 1mm from the slot line, and then by cutting the anode edge approximately 9 cm from this line on the anode side. In order to keep a mark for orienting the gel during the following works, one corner is removed at one specific angle chosen by the operator. Gel is then transferred on a slicing guide (with 1.0 to 1.2 mm flange). Using the gel-slicer, the starch slab of 1cm thick can be cut into 6 thin slices. Each slice obtained is placed in a staining tray, according to a previous chosen order.

The following order can be proposed (slice 1 = bottom of the mould; * obligatory pH)

Slice	Gel pH6	Gel pH8
1	Malate dehydrogenase *	Phosphoglucomutase *
2	Alanine amino peptidase and Leucine amino peptidase	Glutamate oxaloacetate transaminase *
3	Phospho glucose isomerase *	Esterase
4	Phosphatase acid *	Isocitrate dehydrogenase *
5	Phospho Gluconate Dehydrogenase	Alcohol dehydrogenase
6	Diaphorase *	Shikimate dehydrogenase

4.5 Staining

Esterase

The gel slice is pre-incubated during 15 min at room temperature in pH 6.5 phosphate buffer (50ml). The buffer is pourred out and a-Naphtyl acetate /acetone (15ml) with phosphate buffer up to 50 ml is added. Incubate 15 min in the dark at 40°C. The second incubation solution is pourred out and the Fast Blue RR (50 mg dissolved in 25 ml distilled water, in the dark) is added. Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%.

Alcohol dehydrogenase

Incubate the gel slice in the dark at 40°C with the mix 0.5M pH8.5 Tris HCl buffer (10ml), NAD (1ml), MTT (1ml), PMS (1ml), Ethanol 95° (2ml), distilled water up to 50 ml. Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%.

Glucose phosphate isomerase

Incubate the gel slice in the dark at 40°C with the mix 0.5M pH8.5 Tris HCl buffer (20ml), 0.4 M $MgCl_2$ (1ml), NADP (1ml), G6PDH (12.5µl), MTT (1ml), PMS (1ml), fructose 6 phosphate (50mg), distilled water up to 50 ml. Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%.

Phosphoglucomutase

Incubate the gel slice in the dark at 40°C with the mix 0.5M pH8.5 Tris HCl buffer (20ml), 0.4 M $MgCl_2$ (1ml), NADP (1ml), G6PDH (12.5µl), MTT (1ml), PMS (1ml), glucose 1 phosphate (50mg), distilled water up to 50 ml. Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%.

Alanine aminopeptidase and leucine aminopeptidase

The gel slice is pre-incubated during 15 min in pH4.5 Tris Maleate buffer (20ml) with distilled water up to 50 ml. Discard the incubate solution and stain in the dark at 40°C with the mix: Fast Black K (50 mg) dissolved in Tris Malate buffer (20ml), completed with leucine (10ml) and distilled water up to 50 ml. Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%.

Glutamate oxaloacetate transaminase

The gel slice is incubated in the dark at 40°C with the mix: pH8.5 Tris HCl buffer (20ml), aspartic acid (200mg), a-ketoglutaric acid (100mg), EDTA (50mg), PVP (200 mg, M.W. 10 000), distilled water up to 50 ml, completed before complete dissolution with Fast Blue BB (80 mg) and Pyridoxal 5 Phosphate (8mg). Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%.

Malate dehydrogenase

Incubate the gel slice in the dark at 40°C with the mix: pH8.5 Tris HCl buffer (10ml), Na malate (5ml), NAD (1ml), MTT (1ml), PMS (1ml) and water up to 50 ml. Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%.

Isocitrate dehydrogenase

Incubate the gel slice in the dark at 40°C with the mix: pH8.5 Tris HCl buffer (10ml), 0.4 M $MgCl_2$ (1ml), NADP (1ml), MTT (1ml), PMS (1ml), sodium isocitrate (100mg) and distilled water up to 50 ml. Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%. Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%.

Acid phosphatase

Pre-incubate the gel at room temperature in pH 5.0 acetate buffer (15ml), 0.4M $MgCl_2$ (1ml) and distilled water up to 50 ml. Pour out the buffer and add a-Naphtyl acid phosphate (90mg), b-Naphtyl acid phosphate (10mg), Fast Garnet GBC (50mg) dissolved in pH5.0 acetate buffer (15ml), 0.4M $MgCl_2$ (1ml) and distilled water up to 50 ml. Incubate in the dark at 40°C.

Phosphogluconate dehydrogenase

Incubate the gel slice in the dark at 40°C with the mix pH8.5 Tris HCl buffer (10ml), 0.4 M $MgCl_2$ (1ml), NADP (1ml), MTT (1ml), PMS (1ml), 6-phosphogluconic acid (30 mg), and distilled water up to 50 ml. Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%.

Diaphorase

Incubate the gel slice in the dark at 40°C with the mix pH8.5 Tris HCl buffer (10ml), Dichlorophenol indophenol DCPIP (2 ml), MTT (2ml), NADH (20 mg), and distilled water up to 50 ml. Control appearance of coloured bands, rince two times. DO NOT stop staining with acetic acid, but let the slice under water.

Shikimate dehydrogenase

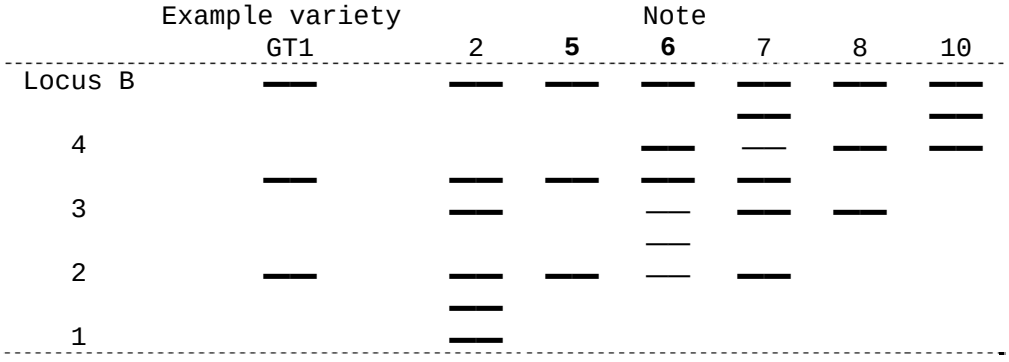
Incubate the gel slice in the dark at 40°C with the mix pH8.5 Tris HCl buffer (10ml), 0.4 M $MgCl_2$ (1ml), NADP (1ml), MTT (1ml), PMS (1ml), shikimic acid (100mg) and distilled water up to 50 ml. Control appearance of coloured bands, rince two times and stop staining with acetic acid 10%.

4.6 Recognition of alleles

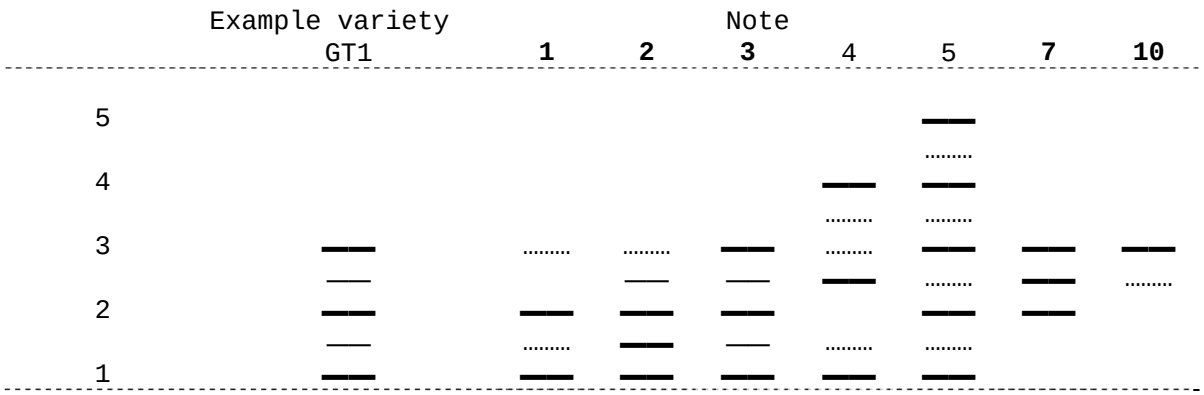
This table is designed to illustrate the alleles described above and to assist in the recognition of the different bands. It depicts the position of all enzyme systems bands, as compared to those found in the Example Variety GT1.

Nomenclature of the individual bands and recognition of the corresponding alleles

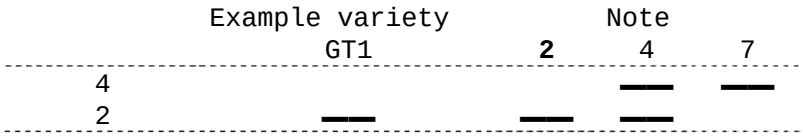
Characteristic 28: **Malate dehydrogenase** locus A



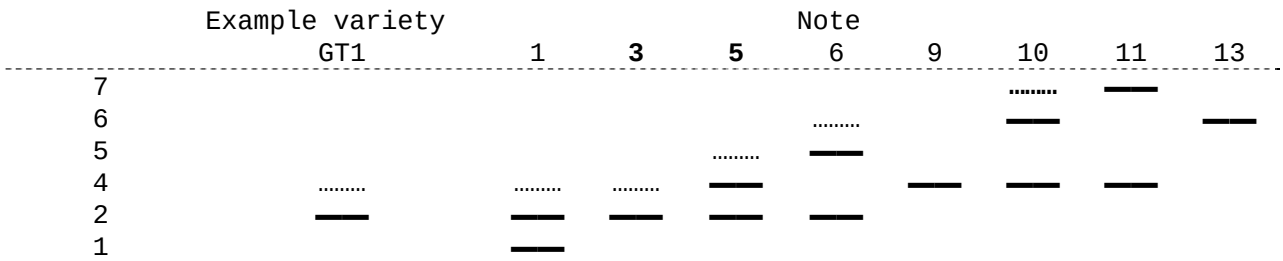
Characteristic 29: **Phospho glucose isomerase** locus



Characteristic 30: **Alanine amino peptidase** locus



Characteristic 31: **Leucine amino peptidase** locus



Characteristic 32: **Esterase** locus

	Example variety			Note		
	GT1	**1**	4	**6**	15	**16**
6				▬	▬	▬
5	.....	.....		.....	▬	.....
4			▬			
1	▬	▬	▬	▬		

Characteristic 33: **Diaphorase** locus

	Example variety		Note	
	GT1	**4**	5	**7**
	──			──
	.....			.....
3	▬	──	▬	▬
	.....	──	──	.....
2	──	▬	▬	──
		──	──	
		──	──	
			──	

Characteristic 34: **Phosphatase acid** locus

	Example variety		Note	
	GT1	**3**	**4**	5
2			──	▬
			▬	
1	▬	▬	──	

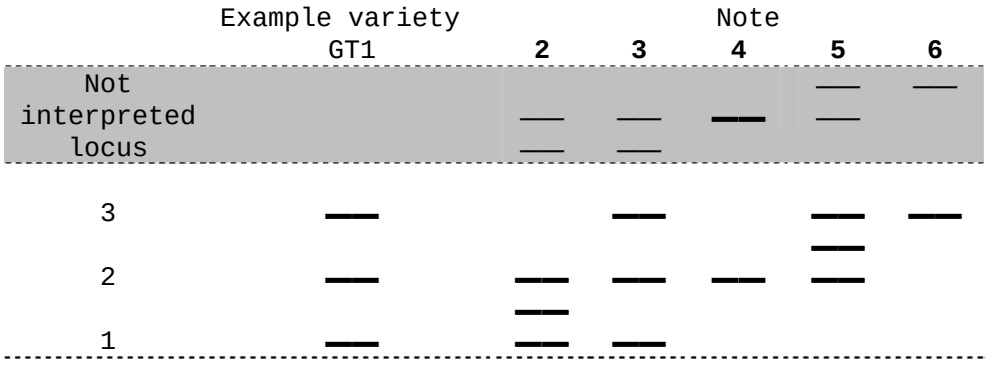
Characteristic 35: **Alcohol dehydrogenase** locus

	Example variety			Note		
	GT1	1	**2**	3	**4**	5
4				▬		▬
						▬
3	▬		▬	▬	▬	▬
			▬			
2		▬	▬	▬		

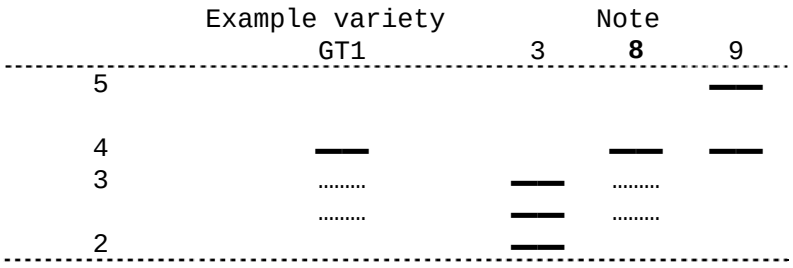
Characteristic 36: **Isocitrate dehydrogenase** locus

	Example variety		Note	
	GT1	**6**	**7**	**8**
			──	▬
4	.....	.....	▬	▬
3	▬	▬	▬	

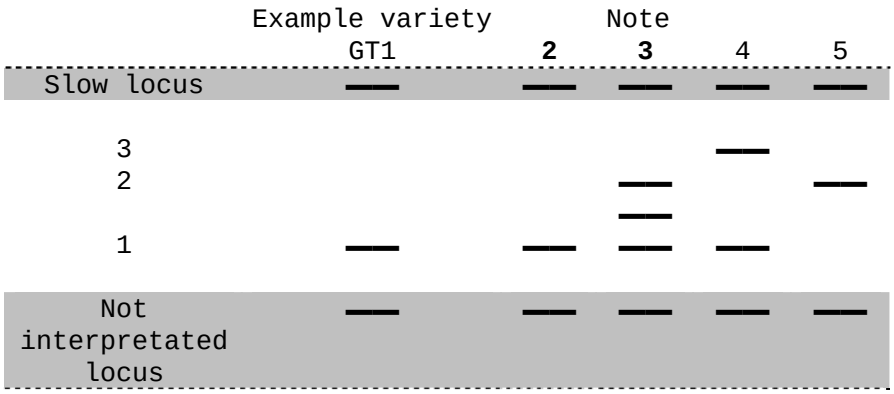
Characteristic 37: **Phosphogluconate dehydrogenase** locus



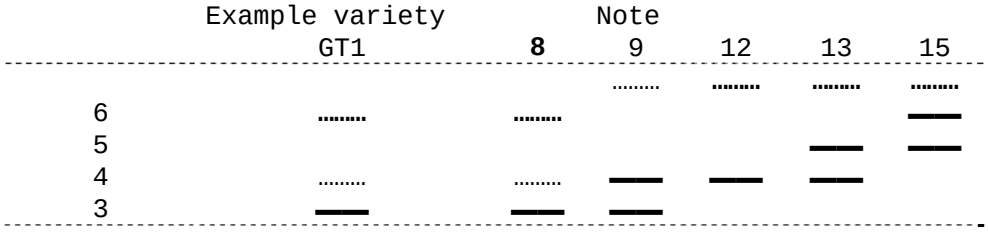
Characteristic 38: **Phosphoglucomatase** locus



Characteristic 39: **Glutamate oxaloacetate transaminase** locus



Characteristic 40: **Shikimate dehydrogenase** locus



Rubber electrophoresis
Chemical supplies references

CHEMICALS (Complete name)	CHEMICAL (Abbreviated form)	SUPPLIER	CATALOG NUMBER
Alpha-NAPHTYL ACID PHOSPHATE monosodium salt	a-NAPHTYL ACID PHOSPHATE	SIGMA	N 7000
Alpha-NAPHTYL ACETATE crystalline	a-NAPHTYL ACETATE	SIGMA	N 8505
DL-ASPARTIC ACID free acid	ASPARTIC ACID	SIGMA	A 9006
MALEIC ACID DISODIUM SALT repurified	MALEIC ACID	SIGMA	M 0375
DL-MALIC ACID free acid	MALIC ACID	SIGMA	M 0875
STARCH POTATO HYDROLYZED FOR ELECTROPHORESIS	STARCH	SIGMA	S 4501
beta-NAPHTYL ACID PHOSPHATE monosodium salt	b- NAPHTYL ACID PHOSPHATE	SIGMA	N 7375
BROMOPHENOL BLUE sodium salt	BROMOPHENOL BLUE	SIGMA	B 8026
L-CYSTEINE free base	CYSTEIN	SIGMA	C 7755
2.6-DICHLOROPHENOL INDOPHENOL sodium salt	DCPIP	SIGMA	D 1878
ETHYLENEDIAMINETETRAACETIC ACID	EDTA	SIGMA	E 5513
FAST BLACK K SALT practical grade	FAST BLACK K	SIGMA	F 7253
FAST BLUE BB SALT practical grade	FAST BLUE BB	SIGMA	F 0250
FAST BLUE RR SALT crystalline	FAST BLUE RR	SIGMA	F 0500
FAST GARNET GBC SALTpractical grade	FAST GARNET	SIGMA	F 0875
D-FRUCTOSE-6-PHOSPHATE disodium salt	FRUCTOSE 6 PHOSPHATE	SIGMA	F 3627
GLUCOSE-6-PHOSPHATE DESHYDROGENASE type XXIII	G6PDH	SIGMA	G 5760
Alpha-D GLUCOSE 1-PHOSPHATE disodium salt, hydrate, crystalline	GLUCOSE 1 PHOSPHATE	SIGMA	G 1259
L-HISTIDINE monohydrochloride: monohydrate	HISTIDIN	SIGMA	H 8125
DL-ISOCITRIC ACID trisodium salt	SODIUM ISOCITRATE	SIGMA	I 1252
Alpha-KETOGLUTARIC ACID free acid, crystalline	KETOGLUTARIC ACID	SIGMA	K 1750
L-LEUCINE beta-NAPHTYLAMIDE hydrochloride, crystalline	LEUCINE	SIGMA	L 0376
MAGNESIUM CHLORIDE hexahydrate	$MgCl_2$	SIGMA	M 0250
DIMETHYLTHIAZOL TETRAZOLIUM	MTT	SIGMA	M 2128
beta-NICOTINAMIDE ADENINE DINUCLEOTIDE	NAD	SIGMA	N 3014
beta-NICOTINAMIDE ADENINE DINUCLEOTIDE reduced form , disodium salt	NADH	SIGMA	N 6005
beta-NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE sodium salt	NADP	SIGMA	N 0505
6-PHOSPHOGLUCONIC ACID trisodiumsalt , grade III, crystalline	PHOSPHOGLUCONIC ACID	SIGMA	P 6888
PHENAZINE METHOSULFATE	PMS	SIGMA	P 9625
POLYVINYL PYRROLIDONE (M.W. 10 000)	PVP P.M. 10 000	SIGMA	PVP 10
POLYVINYL POLYPYRROLIDONE	PVPP	SIGMA	P 6755

CHEMICALS (Complete name)	**CHEMICAL (Abbreviated form)**	**SUPPLIER**	**CATALOG NUMBER**
PYRIDOXAL 5-PHOSPHATE	PYRIDOXAL 5 PHOSPHATE	SIGMA	P 9255
SHIKIMIC ACID	SHIKIMIC ACID	SIGMA	S 5375
SODIUM ACETATE ANHYDROUS	SODIUM ACETATE	SIGMA	S 8750
TRIS (TRIZMA BASE)	TRIS	SIGMA	T 1503
TRITON X100 laboratory grade	TRITO X100	SIGMA	X 100
ACETONE	ACETONE	MERCK	14 1000
ACETIC ACID	ACETIC ACID	MERCK	62 1000
CHLORYDRIC ACID	CHLORHYDRIC ACID	MERCK	317 1000
CITRIC ACID MONOHYDRATE	CITRIC ACID	MERCK	214 1000
ETHANOL	ETHANOL	MERCK	983 1000
POTASSIUM DIHYDROGENOPHOSPHATE	KH_2PO_4	MERCK	48731000
SODIUM HYDROGENOPHOSPHATE	Na_2HPO_4	MERCK	6580 0500
SODIUM HYDROXYDE	NaOH	MERCK	6498 1000
GLYCEROL (TECHNICAL GRADE)	GLYCEROL	LABOSI	G 350

[End of Annex and of document]